AUTOPSY INVESTIGATION IN STILLBIRTH

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I, Julie Anne Man, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed:

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Abstract

The UK has the highest rate of stillbirth in the developed world and there are more than three million stillbirths worldwide each year. With over 30 different classification systems, the rate of unexplained stillbirth varies from 15-60% despite postmortem investigation being undertaken in a number of cases. The primary aims of this project were to use a unique autopsy database to examine a large well characterised series of stillbirth autopsies to assess specific causes of death, relationships between fetal maceration, intrauterine and postmortem interval on cause of death and fetal intrauterine growth restriction as well as producing evidence based guidelines for autopsy practise and investigating the potential role of novel techniques in future stillbirth autopsy.

The analysis of more than 1000 intrauterine and intrapartum fetal deaths revealed that two thirds had an unexplained cause of death. Internal examination of the fetuses provided a definitive cause of death in 1% of cases; 19% of the overall causes of death could have been diagnosed from review of the clinical circumstances and a further 18% by placental macroscopic and microscopic examination. Significant associations were found between increasing maceration and Small for Gestational Age (SGA) fetuses and that using birthweight or bodyweight alone erroneously overestimate the role of SGA as an underlying factor in stillbirth causation. Other investigations such as modified organ weight ratios may contribute to determining cause of death. Proteomic experiments proved that in principle significantly different amounts and types proteins could be successfully extracted from formalin fixed paraffin embedded stillbirth fetal tissue in different case groups, suggesting a possible future investigation into the causation of stillbirth.

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1.0 Background

The stillborn (definition) Act 1992 defines stillborn as a child that has issued forth from its mother after the 24th week of pregnancy and that did not at any time after being completely expelled from its mother breathe or show any signs of life (1). Intrauterine death can be defined a fetus with no sign of life in utero (2).

In 2012 there were 729,674 births in England and Wales of which 3,558 were stillborn, a rate of 4.9 stillbirths per 1000 births (3). Depending on which classification system is used, 15-60% of all stillbirths remain unexplained, despite postmortem examinations being undertaken by trained professionals in a number of cases (4, 5). Stillbirth is 10 times more common than sudden infant death syndrome and is nearly five times more common than infant deaths related to congenital anomalies (6).

Globally, stillbirth has a large public health impact and specific causes of death include congenital abnormalities, ascending infection, placental abruption, placental insufficiency and complications of labour (5, 7-28). There are an estimated 1.02 million intrapartum stillbirths each year and a total of at least 3.2 million stillbirths worldwide although these numbers may be underestimates due to under-reporting, and inconsistent definitions (29-32). The majority of stillbirths occur in low to middle income countries and approximately half occur at home, causing the deaths to be uncounted and unnamed (6, 33-43). Household surveys are often the only way of collecting data on child mortality; however their expense limits the number of reviews to one every five years in most countries making their ability to look at rapid change in mortality limited (33). Verbal autopsy methods are used to collect data through the use of questionnaires that are given to the remaining family members

after the death of the fetus (13, 34). Data is often not collected in those fetal losses under 28 weeks gestation in most developing countries or in those fetuses that weigh less than 1000g further limiting data collection (6).

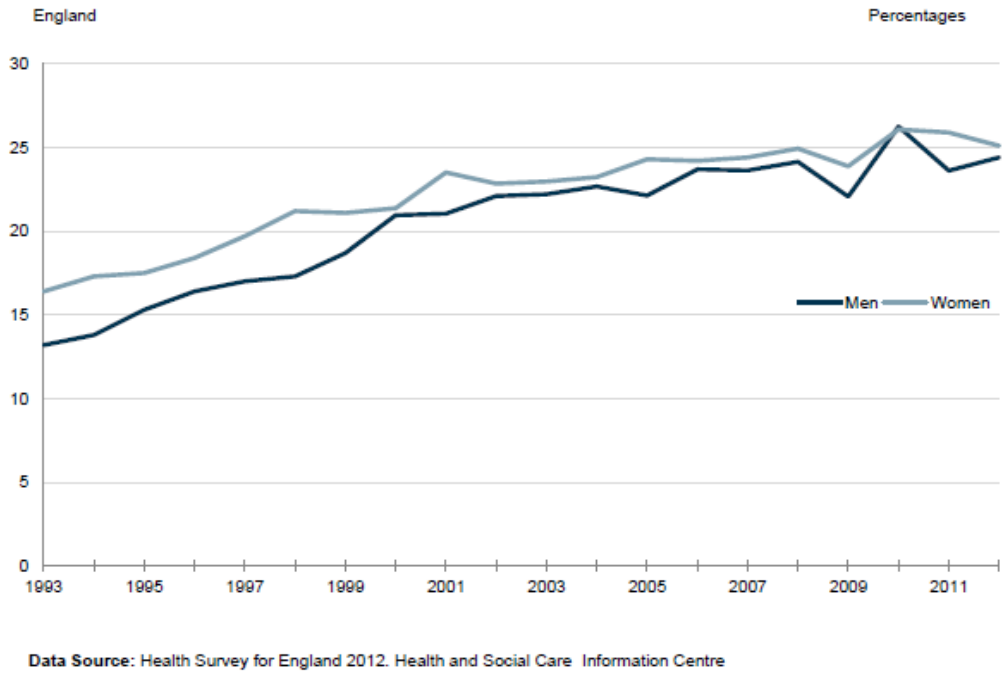

Figure 1 *Obesity prevalence in Adults (16 years+) 1993 – 2012. Copyright © 2016, Re-used with permission of the Health and Social Care Information Centre. All rights reserved. (44)*

Epidemiological associations include a parity of zero or greater than 3; maternal ethnicity (particularly black mothers), consanguineous marriage, advanced maternal age and maternal obesity (45-60). These findings are worrying since the national rate of obesity in the United Kingdom is rising (44) (Figure 1). Other associations include smoking; maternal Diabetes Mellitus; maternal hypertension; history of antenatal vaginal bleeding, maternal cholestasis and fetal intrauterine growth restriction (IUGR) (48, 49, 51, 52, 61-82). It has been suggested that unrecognised intrauterine growth restriction may be a major contributing factor, suggesting that some deaths of normally formed stillborn fetuses could be avoided if fetal growth disturbances were detected and interventions performed. 75% of fetuses with intrauterine growth restriction are not recognised until after delivery and this rises to

85% in low risk pregnancies. The majority of our data on 'IUGR' comes from surrogate data based on small for gestational age (SGA) fetuses, assessing the weight of liveborn infants or stillborns (83-85).

Awareness of the improvement in detecting SGA antenatally, of which a proportion will be IUGR, is now recognised (86-92). Using a birthweight of $<10^{th}$ percentile for the diagnosis of SGA and taking into account maternal ethnicity, weight, parity, gestational age of the fetus and fetal gender, one study reported that 52% of the stillbirths were SGA (93).

Studies have also highlighted the role of ethnicity and stillbirth, reporting that Black and Asian women have twice the odds of stillbirth compared to white women even after adjusting for age, parity, deprivation, obesity, hypertension and diabetes; mothers who were native to South Asia also have an increased risk of antepartum stillbirth in late pregnancy (37 -42 weeks gestation) compared to other women in the Australian population (94, 95).

Maternal Age is a risk factor for fetal loss with women between the ages of 30-34 having a 27% greater risk compared to mothers between the ages of 25-29, after adjusting for parity, ethnicity, body mass index, maternal co-morbidities and deprivation (96). Women aged over 40 years at the time of delivery also have an increased risk of stillbirth (RR 1.83), independent of parity and adjusting for socioeconomic status (97).

The day of a person's birth is the day of ones greatest risk of death until one reaches old age but despite this and all the associated risk factors discussed above, stillbirths are not currently recorded in the Millennium Development Goal (MDG) for child survival or the Global Burden of disease metrics which is also likely to be another

reason for the underestimation of the total number of stillbirths worldwide (29, 30, 98).

In 2011 a series of high profile papers was published which highlighted the global burden of stillbirth. 'Stillbirths: why they matter' presented a survey of 2,490 health professionals in 135 countries and 1,127 parents in 32 countries (99). This highlighted several important issues. Mothers who had experienced a stillbirth were often subjected to stigma and marginalisation in communities that would blame the death on the mothers own sins, evil spirits and destiny with little bereavement support, particularly in Sub-Saharan Africa (99). One in two mothers were unable to grieve in public due to stigma attached to stillbirth and this experience was not limited to Africa but was also found throughout parts of Europe, Russia and South America (Figure 2)(99).

Figure 2 *Percentages of stigma felt by grieving mothers of stillborn infants (Permission to reproduce this figure has been granted by Elsevier through Copyright Clearance Centre) (99)*

Low income countries, showed a much higher degree of detachment to the stillborn fetus than more developed countries with the overall survey finding that four out of every five fetuses that were stillborn had been disposed of without a name and without any ritual recognition of the passing of life; 75% had not been held or

dressed and one quarter were not seen by the mother or family members (99). A disparity in stillbirth rates globally was found with an inverse association with sex equality such as secondary education and reproductive control (Figure 3) (99).

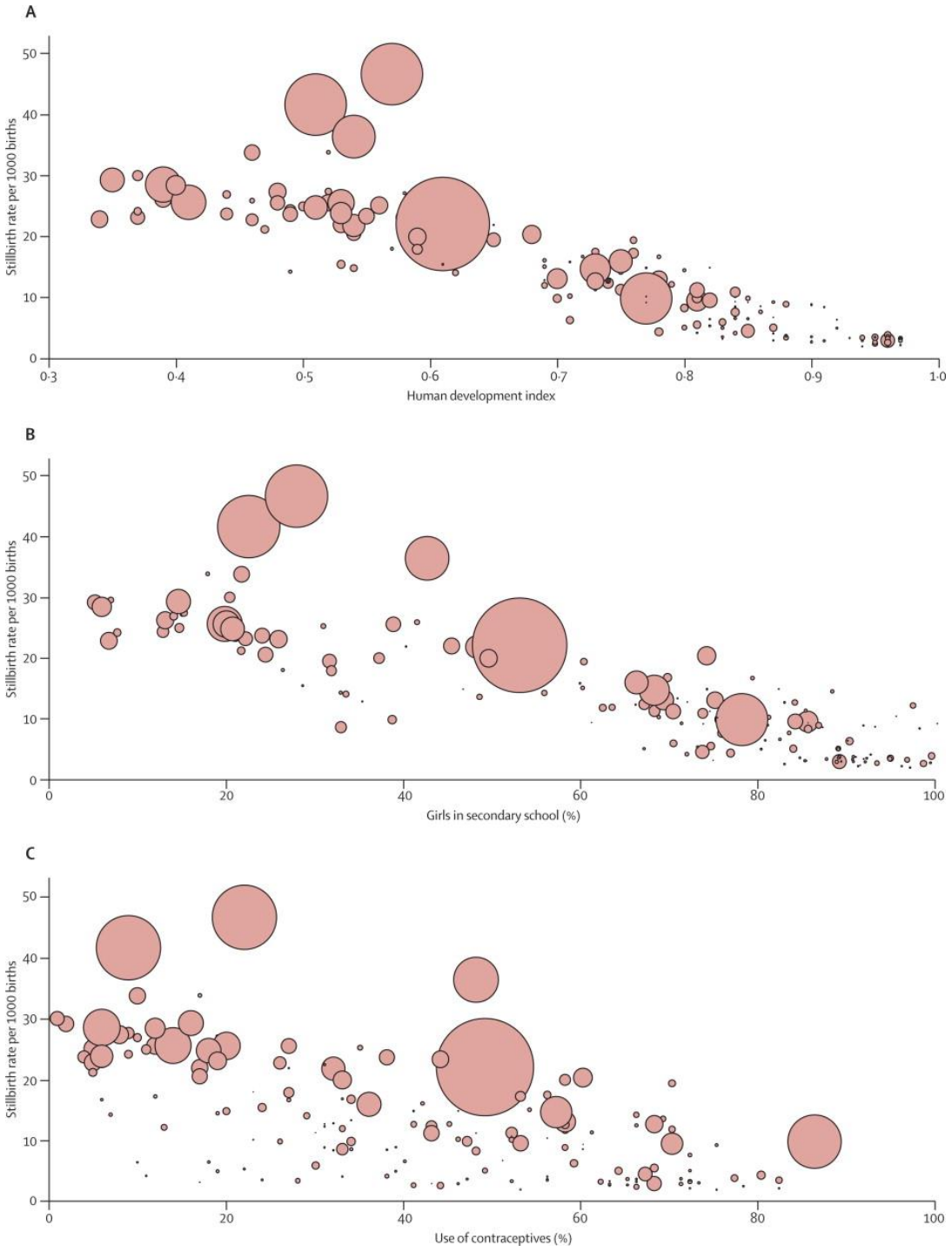

Figure 3 *Stillbirth rates plotted against indicators of disparity in income and sex equality (Permission to reproduce this figure has been granted by Elsevier through Copyright Clearance Centre) (99)*

Seventy percent of those people completing the survey in high burden areas agreed that stillbirth prevention needed prioritising (99). A lack of knowledge on the causes of stillbirth combined with limited evidence of preventability means that much more data collection on the rates and causes of stillbirths together with any preventable strategies is needed in order to convince more health professionals that stillbirth should be as highly prioritised as preventing infant and maternal deaths (99). The lasting message was that stillbirths need visible leaders for both local communities and on the wider international platform to encourage the United Nations to include stillbirth in their global health policy (99).

'Stillbirths: Where? When? Why? How to make the data count', highlighted the invisibility of stillbirth; how stillbirth is not on the Millennium Development Goals, or in the Global Burden of Disease metrics, as fore mentioned nor is data compiled by the United Nations (100).

'Stillbirths: what difference can we make and at what cost' was a systematic review of randomised trials and observational studies of interventions which could reduce the burden of stillbirth, particularly in low and middle-income countries (101). 98% of the 2.65 million stillbirths worldwide occur in low-middle income countries (101). The review found that the main causes of stillbirth in these settings were; childbirth complications, maternal infections in pregnancy (e.g. syphilis), maternal disorders (particularly hypertension), maternal under nutrition, fetal growth restriction and congenital abnormalities. The authors reviewed 35 possible interventions that may reduce stillbirth, 10 of which were highly recommended by the paper for implementation. These included; periconceptional folic acid fortification, insecticide –treated bed nets or intermittent preventative treatment for malaria prevention, syphilis detection and treatment, detection and management of hypertensive disease

of pregnancy, detection and management of diabetes in pregnancy, detection and management of fetal growth restriction, routine induction to prevent post-term pregnancies, skilled care at birth, basic emergency obstetric care and comprehensive emergency obstetric care (101).

In high income countries, less than 10% of all stillbirths are intrapartum and there is good access to high quality care compared to low and middle income countries where there are an estimated 46% of women giving birth without skilled assistance (101, 102). Using the Lives Saved Tool the authors found that if there was 99% use of these 10 recommendations, 45% of stillbirths could be prevented (101). The authors finally highlighted the need for further data collection on the gestational age of the stillbirth and whether the death was ante or intrapartum- a similar plea to that of the first stillbirth series paper (99, 101).

'Stillbirths: how can health systems deliver for mothers and babies' focused on how the recommendations outlined above could be implemented and 'Stillbirths: the way forward in high income countries' highlighted that the UK has one of the highest stillbirth rates in high income countries, recognises that there are modifiable risk factors for stillbirth and suggests the need for further data collection and a thorough investigation of the death is necessary including placental histopathology and autopsy (103, 104). The authors agree that there needs to be a consensus on the definition and classification of stillbirth in order to further our knowledge and data collection together with research into the underlying mechanisms of stillbirth and how it can be detected; thoughts echoed by other literature (104-107).

In the United Kingdom, SANDs, the stillbirth and neonatal death charity, shares the thoughts above and offers support to those affected by the death of a baby. The

charity aims to promote research into the field and helps to fund research programmes such as this project (108). The charity was founded in 1978 by two bereaved parents (108). Over the course of the last 30 years the Charity has made great progress in the recognition of the grief caused by stillbirth and the medical and legal documentation of the death and postmortem in the UK. (108).

1.1 Classification systems for perinatal deaths and stillbirth

As highlighted in the Lancet series, the classification of stillbirth varies globally as do the terms used to describe it such as birth asphyxia, fresh stillbirth, intrauterine stillbirth and delivery-related perinatal death, making the collation of data more difficult (104, 107, 109, 110).

Over the last 60 years there have been many attempts to classify stillbirths. In 1954 the Aberdeen classification system was developed after stillbirths first became notifiable in Scotland in 1940 (111, 112). Figure 4 displays the Aberdeen classification of stillbirth which is based more on obstetric findings and complications than findings at postmortem (113).

OBSTETRIC (Aberdeen) CLASSIFICATION

Categories at the head of the list take priority over those lower down. Only ONE answer applies – it is the lowest numbered category that adequately describes the death.

Code	Category
	Congenital anomaly:- any structural or genetic defect incompatible with life or potentially treatable but causing death
1	Neural tube defects
2	Other anomalies
	Isoimmunisation:- death ascribable to blood group incompatibility
3	Due to Rhesus (D) antigen
4	Due to other antigens
	Pre-eclampsia
5	Without APH
6	Complicated by APH
	Antepartum Haemorrhage (APH)
7	With placenta praevia
8	With placental abruption
9	APH of uncertain origin
	Mechanical
10	Cord prolapse or compression with vertex or face presentation
11	Other vertex or face presentation
12	Breech presentation
13	Oblique or compound presentation, uterine rupture etc.
	Maternal disorder
14	Maternal hypertensive disease
15	Other maternal disease
16	Maternal infection
	Miscellaneous
17	Neonatal infection
18	Other neonatal disease
19	Specific fetal conditions
	Unexplained
20	Equal or greater than 2.5kg
21	Less than 2.5kg
22	Unclassifiable

Figure 4 *Aberdeen classification of stillbirth (113)*

In 1980, the Wigglesworth classification system was created in an attempt to simplify and unify professional's opinions about cause of death with the purpose of this classification to subdivide cases into groups with clear implications for clinical management (4).

The Wigglesworth system is based on major groups of pathophysiological abnormalities/conditions of the fetus defined as;

1. Normally formed macerated (Stillbirth)
2. Congenital malformation (Stillbirth or neonatal death)
3. Conditions associated with immunity (Neonatal death)
4. Asphyxial conditions developing in labour (Fresh stillbirth/neonatal death)
5. Specific conditions other than the above (4)

This classification system has several flaws. Firstly, it covers both stillbirths and neonatal deaths, two separate entities. Secondly a normally formed but macerated fetus is not a cause of death and this classification does not help to distinguish those fetuses that died before or during labour (4). The classification system may help clinicians understand areas of deficit within their clinical practise, but it does not provide a pathological cause of death.

In 2005 an article was published on a further classification system for stillbirth, ReCoDe an acronym for Relevant Condition at Death (5) (Figure 5). The aim of this system was to establish what conditions are associated with death not necessarily the mechanisms(5). It classifies fetal growth restriction as a fetus below the 10th customised centile (5). Using registry data of over 2500 cases of stillbirths over a 7 year period in the UK, the study demonstrated that when the Wigglesworth classification system, the fetal and neonatal classification system and the revised obstetric (Aberdeen) classification systems were used, the cause of death was unexplained in 66.2%, 66.2% and 52.7% respectively.

Using the ReCoDe classification, only 15.2% of cases were unexplained and the majority (43%) fell into the category of fetal growth restriction (5). This study highlighted the need for further investigation into the association between fetal growth restriction and stillbirth, particularly when 57.7% of Wigglesworth's unexplained cases were found to be growth restricted in the ReCoDe study (5).

The ReCoDe system appears more comprehensive

Classification system according to relevant condition at death (ReCoDe)

Group A: Fetus
1. Lethal congenital anomaly
2. Infection
2.1 Chronic
2.2 Acute
3. Non-immune hydrops
4. Isoimmunisation
5. Fetomaternal haemorrhage
6. Twin-twin transfusion
7. Fetal growth restriction*

Group B: Umbilical cord
1. Prolapse
2. Constricting loop or knot†
3. Velamentous insertion
4. Other

Group C: Placenta
1. Abruption
2. Praevia
3. Vasa praevia
4. Other "placental insufficiency"‡
5. Other

Group D: Amniotic fluid
1. Chorioamnionitis
2. Oligohydramnios†
3. Polyhydramnios†
4. Other

Group E: Uterus
1. Rupture
2. Uterine anomalies
3. Other

Group F: Mother
1. Diabetes
2. Thyroid diseases
3. Essential hypertension
4. Hypertensive diseases in pregnancy
5. Lupus or antiphospholipid syndrome
6. Cholestasis
7. Drug misuse
8. Other

Group G: Intrapartum
1. Asphyxia
2. Birth trauma

Group H: Trauma
1. External
2. Iatrogenic

Group I: Unclassified
1. No relevant condition identified
2. No information available

* < 10th customised weight for gestational age centile.
†If severe enough to be considered relevant.

Figure 5 *ReCoDe Stillbirth Classification (Permission to reproduce this figure has been granted by BMJ Publishing Group Ltd) (5)*

but does not necessarily address why the fetus died or what went wrong and has a major emphasis on fetal weight as a defining factor for IUGR, which is likely erroneous (See Chapter 5).

In 2009, Causes Of Death and Associated Conditions, classification was published (114). CODAC was developed to follow the concepts within the International Classification of disease (ICD) and contains ten main categories; intrapartum events, infections, congenital abnormalities, unknown causes of death, termination of pregnancy, fetal, cord, placental, maternal, conditions relevant to the neonatal period (114).

In full, CODAC contains 94 subcategories which are then further specified into 577 categories (114). The system is designed to accommodate both the main cause of death as well as two associated conditions and combines both the clinical and pathological causes of stillbirth (114).

A review of six stillbirth classification systems, the Amended Aberdeen, the Extended Wigglesworth, PSANZ-PDC (Perinatal Society of Australia and New Zealand – Perinatal Death Classification), ReCoDe, Tulip and CODAC reported that three of the systems (Tulip, Wigglesworth and Aberdeen) focussed on identifying a single underlying cause of death; CODAC and PSANZ-PDC tried to find the cause of death and any associated conditions on secondary and tertiary levels; and ReCoDe aimed to find a relevant condition and /or a cause of death (115). Only the ReCoDe system was designed for exclusive use in stillbirths (115). Three measures of outcome were used (*InfoKeep:* A scoring system to measure the extent to which the classification teams agreed that important information to aid in the understanding of the death was conserved and retrievable after classification ; *Ease Score:* which

measured the extent to which the classification team agreed that it was easy to identify the relevant category in the classification system; *Inter-observer reliability:* inter-observer agreement beyond chance for the main classification categories was assessed using the unweighted kappa statistic (115)).

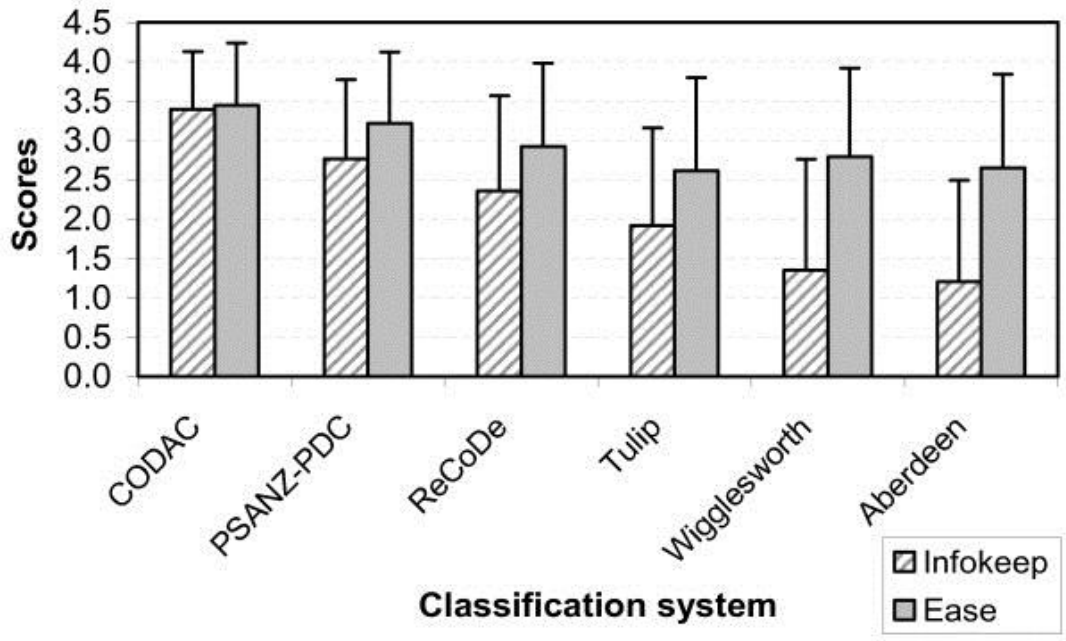

Figure 6 Retention of important information (***InfoKeep***) and ease of use (***Ease***) scores by classification systems. (Permission to reproduce this figure has been granted; the article is an open access article with unrestricted use of its content) (115)

Teams assessed 857 cases of stillbirth (115). *Infokeep* scores were significantly different across the classifications ($p<0.01$; Figure 6) due to low scores in both the Wigglesworth and Aberdeen classifications. The *Ease of use* scores were similar and significantly different across the classifications ($p<0.01$) with CODAC having the highest score and Wigglesworth the lowest (115).

The Aberdeen and Wigglesworth classifications had the worst inter-observer reliability with kappa values of 0.35 and 0.25 respectively. Tulip had the best inter-observer reliability with a kappa value of 0.74 (115).

The proportion of unexplained stillbirth deaths varied greatly between the classification systems but was not statistically significant (p=0.38). The CODAC system had the least amount of unexplained deaths and the Wigglesworth and Aberdeen systems had the most unexplained deaths (115).

This review suggested that the Extended Wigglesworth and the Amended Aberdeen classification systems should no longer be used to classify stillbirths (115). The best performing system was CODAC (115). No one system was a perfect fit and their correct and appropriate use in both developing and developed countries remains a challenge.

Perinatal pathologists, paediatricians, obstetricians and public health professionals need a united classification system that can be used throughout the world simply, effectively, confidently and accurately to continue to improve research into the cause of stillbirth. Furthermore, it should be recognised that different classification systems may be required for different purposes; for example, the assessment of underlying pathophysiological mechanisms versus identifying deficiencies in clinical care.

1.2 The importance of the placenta

The placenta and the umbilical cord are essential for intrauterine life and therefore, unsurprisingly, each classification system discussed above has a section available for causes of death associated with or caused by abnormalities of the placenta (116). Abnormalities at a macroscopic, microscopic and/or molecular level (including infections) within the arteries, veins, villi or umbilical cord can fatally disrupt blood and nutrient flow to the developing fetus (116-137). Abnormalities can include, ascending genital tract infections in which firstly there is a Maternal Inflammatory Response (MIR) followed by a Fetal Inflammatory Response (FIR) although a FIR is not always seen in early preterm stillbirth (138, 139). The innate immune system

responds with neutrophils (140). In the FIR leukocytes migrate from fetal vessels in the umbilical cord to the chorionic plate (funisitis). FIR increases with gestational age and the severity of MIR (141). Histologically ascending genital tract infection is associated with polymorphonuclear leucocytic infiltration of the extraplacental membranes, the intervillous space and finally the chorionic plate (142). Thrombi can form in chorionic vessels due to the activation of endothelial cells which can embolize into the fetus (142). Exposure to infection can cause preterm delivery, hypoxia and death (142, 143). Abnormalities can present secondary to haematogenous infections such as Toxoplasmosis and cytomegalovirus (144).

Hypertension and Pre-eclampsia/Eclampsia can also lead to placental abnormalities of which the former affects around 5-8% of pregnancies (144, 145). A large population based study of 57 million singleton live and stillbirths found that pregnancy induced hypertension is associated with increased risk of stillbirth with a higher risk in second order plus pregnancies than in mothers who are primigravida (145, 146). Pre-eclampsia can be defined as maternal endothelial dysfunction with clinical hypertension, oedema and proteinuria and is associated with increased risk of stillbirth (117, 147).

Placental Abruption, defined as the complete or partial separation of a normally implanted placenta before delivery can cause fetal death, severe haemorrhage, need for blood transfusions, emergency hysterectomy, disseminated intravascular coagulopathy and renal failure and is a recognised cause of stillbirth, especially in the third trimester (148).

Specific pathologies only diagnosable on histological examination associated with growth restriction and fetal death, such as massive perivillous fibrin deposition, can also occur as well as the spectrum of changes associated with maternal vascular malperfusion including those previously known as uteroplacental vascular disease (149, 150).

Placental abnormalities are therefore believed to be a common cause of death in stillbirth, (151). A review of placental pathology in association with stillbirth reported that the placental pathology was associated with stillbirth in anywhere from 11 - 65% of cases. Placental abruption was the most frequently identified placental cause of stillbirth. Diagnostic criteria and nomenclature for placental abnormalities varied between studies thus making observations rather subjective and increasing the risk of observer bias and inter-observer variability (152). Again the lack of an adequate evidence base for interpretation of histological findings is evident together with a lack of any definitive and accurate classification of the cause of death of stillbirth.

At present there are no clinically useful first or second-trimester tests of placental function to reliably predict stillbirth, although uterine artery Doppler indices and maternal serum PAPP-A levels appear to be promising candidates for early detection of placental dysfunction (153). Placental examination in stillbirth autopsy is advised and histological analysis has clinical value, however; improved communication between scientists, clinicians and pathologists and advanced research is still needed to explore further the role of the placenta in stillbirth (154-156).

Cord insertion and placental disc shape, in livebirths, have been reviewed in large scale studies and it is reported that the cord is more typically eccentric than central

and the placental disc elliptical rather than round. No differences were seen comparing groups of normal pregnancies to those with pre-eclampsia, pregnancy induced hypertension, gestational diabetes mellitus or small for gestational age as well as those with specific placental histological abnormalities (157, 158). However, such studies have not been completed in relation to or in comparison to stillbirth.

1.3 Current postmortem investigation is stillbirth

There are currently no evidence based guidelines in the United Kingdom of the pathological investigation of stillbirth; recommendations from the Royal College of Pathologists exist, which are based on expert opinion (159). There are no statutory requirements to investigate intrauterine death and the parents of a stillborn infant can either consent to a full or limited autopsy or decline autopsy examination.

Autopsy uptake after stillbirth has been falling in the UK over the last 10-20 years and now ranges from only 40-50% (160). The discussion between the health professional and parents regarding autopsies usually only occurs once, several hours after the stillbirth, through verbal communication with little written information about autopsy being available to parents (160). It has been recognised that emotional distress and perceived lack of rapport between the health professional and the family may be barriers in gaining consent for stillbirth autopsy together with the health professionals own perception of autopsy; a lack of appropriate training to gain parental consent for autopsy (particularly in cases with cultural diversity) and the history of organ retention by some hospitals (160-162). Parents may refuse autopsy in fearing that their child will be mutilated by the procedure; that they want their child to rest peacefully after its death; and sometimes religious and cultural beliefs about autopsies and the need to bury the dead quickly mean parents decline autopsy (162-165). Improved training and the use of more senior health professionals (i.e.

Consultant Obstetricians rather than juniors or midwives) and the support and availability of perinatal pathology services appear to help improve stillbirth autopsy uptake (166).

Full autopsy consists of an external and an internal examination with the weighing and sampling of organ tissue and the placenta for histological analysis.

Evidence of fetal maceration, the process of fetaltissue changes due to immersion in fluid after intrauterine death, has rarely been investigated or analysed in the context of cause of death in stillbirth, but is frequently commented on during external examination of a fetus at autopsy (167). Maceration can be defined as mild, moderate or severe based on the degree skin slippage/peeling, effusions within serous cavities and fetal mummification but little is reported about any effects of maceration on the overall weight of a fetus, individual organ weights or the ability to assign a cause of death (167). Only one previous study has reported that weights of the liver, thymus and spleen were markedly lighter with increasing maceration in perinatal autopsy cases (168). It is assumed that maceration worsens the longer the time period between intrauterine death and delivery (Intrauterine interval, IUI) but this is based on conjecture highlighting the necessity of assessing both maceration and IUI.

Autopsies are also not completed immediately after death and postmortem interval (PMI) can vary from one day to several weeks, depending on individual circumstances. The effects of postmortem interval on fetal weight, organ weight or cause of death have not been previously systematically investigated.

Fetal brain to liver weight ratio (in grams) is used in clinical pathology practise as a marker for fetal growth restriction. However, this is based on two small historical

studies, neither of which provide data on pathological mechanism of death or possible effects of maceration, IUI or PMI (169, 170).

Histological examination of fetal tissue samples for a wide range of organs is also recommended in Royal College guidance however, there are no studies which have examined in any detail, the usefulness of such histological examination or the correlation between positive histological findings and cause of death. Thymus histology is recommended in stillbirth autopsies to assess for accelerated involution which has been suggested to be an indicator of prenatal stress and IUGR (171, 172). Involution is graded using the Van Baarlen Grading scale, however this assessment is essentially subjective and relies on consistent reporting from perinatal pathologists making judgements on the degree of lymphodepletion and shrinkage of the cortex, without the use of measurements, with great potential for bias (173).

Other investigations such as fetal radiographs, Magnetic Resonance Imaging (MRI) Computered Tomography (CT) and microbiological and virological sampling and are also recommended based on expert opinion (159, 174).

Genetic testing is also recommended, in the appropriate setting, at stillbirth autopsy, however, antenatal ultrasound scanning has greatly advanced over the last 20 years and now plays a vital role in detecting fetal congenital abnormalities, particularly in the second trimester (175). Many of these cases may now be terminated by parental choice thus limiting the need for genetic analysis and confirmation of results at autopsy, however autopsy can provide additional information in up to 30% of these cases (175).

If congenital abnormalities are discovered macroscopically at autopsy the reporting pathologist will usually ask for genetic analysis of fetal tissue. At present

cytogenetics and karyotyping can be undertaken on a fresh placental sample ($1cm^3$) from the fetal aspect of the placenta and thus needs to be collected prior to the placenta being placed in formalin (175, 176). DNA from organ tissue samples can also be helpful and important as karyotyping can differ between the fetus and its placenta: DNA can still be extracted from such fresh tissue 3-5 days after delivery (175, 176). Abnormal fetal karyotypes have been reported in stillbirths at similar proportions to liveborns, the majority being 45X, trisomy 21, trisomy 18 and trisomy 13, however standard karyotyping can fail in up to 50% of cases and cannot detect abnormalities less than 5 megabases (176). New laboratory techniques such as Comparative Genomic Hybridization (CGH), which does not rely on tissue culture and appears to have a higher success rate than conventional karyotyping, could lead to greater detection of genetic abnormalities in stillbirth due to its ability to evaluate the whole genome for microdeletions 1 megabase or smaller (176-178). However, CGH may be more useful in detecting abnormalities in miscarriage than stillbirth with detection rates as low as 13% in specific stillbirth populations with known congenital abnormalities and CGH is still significantly more expensive than conventional karyotyping (179-181).

It is only recently been hypothesized that single gene mutations (particularly in NOS, HIF-1, Klotho and TNF – alpha) and missense mutations associated with LQTS susceptibility may account for a proportion of currently unexplained stillbirths (182, 183). Initial studies have found no significant data in regards to single gene mutations but a small percentage (3.3%) of a selection of unexplained fetal deaths (91 cases) had missense mutations associated with LQTS susceptibility and 8% of cases had genetic variants leading to dysfunctional LQTS-0 associated ion channels in vitro, suggesting there may be a cohort of unexplained stillbirths with genetic

abnormalities that could directly or indirectly lead to death (182, 183). More research is required in this area with a greater cohort of patients to further establish the role and importance of genetics in unexplained stillbirth.

1.4 The project

The primary aims of this project are therefore to use a unique autopsy database with pre-defined and objective criteria for all fields to examine a large well characterised series of stillbirth autopsies to:

1. Analyse specific causes of death in stillbirth, including in depth analysis of death associated with specific and non-specific placental pathology
2. Assess for possible relationships between fetal maceration, intrauterine interval and postmortem interval on body and organs weights with detailed analysis of fetuses found to be IUGR/ SGA
3. Assess the clinical usefulness of histological analysis of fetal tissue
4. Review and analyse the role of thymic involution in stillbirth autopsy practise
5. Investigate the potential role of new novel techniques in future stillbirth autopsy investigation
6. Produce evidence based guidance for stillbirth autopsy practise.

This is the largest study of its kind and the only one in which, rather than using autopsy findings based on subjective interpretation, all data fields have predefined, objective criteria with pathological findings clearly distinct from interpretation. In addition to providing an evidence base for practice, it will identify areas for further research in stillbirth.

2. Methods

2.0 The Database

2.1 Modifications to the database

2.2 Data Entry

2.3 Histology and Laboratory Work

2.0 The database

During previous research at Great Ormond Street Hospital an objective Microsoft Access Database, named the Rapid Study Database was designed for the input of postmortem data in cases of Sudden Unexpected Infant Death (184). Although extensive the database did not have areas designed for the input of specific details such as antenatal and delivery history, which are vital when investigating stillbirth. Modifications to the database were therefore required in order to collect and analyse data from stillbirth and miscarriage postmortems.

2.1 Modifications to the database

Eight new tables were created and added to the existing database to improve data entry in the most objective manner possible (*Table 1*). Modifications to the database took two months. *Appendix 1* describes in detail, the process of creating the new tables and intergrating them as forms into the rapid study database. After modifications there were over 400 fields of entry for each case in the database.

New Table and Form heading	Details entered in the form
Antenatal Details	• Maternal blood tests • 12 and 20 week Ultrasound scan report findings including Doppler results. • Space to record any evidence of pre-eclampsia, Diabetes Mellitus and other ultrasound scan reports. • Maternal body mass index • Gestation of intrauterine death. • Free text box for any further information
Delivery Details	• Mode of delivery • If caesarean, why this was undertaken • Details of any intrapartum events

	• Degree of fetal maceration
Placenta	• Type of placenta (single or multiple) • Placental weight (trimmed) • Cord length, vessels and coiling • Macroscopic and microscopic description for cord, membranes and placental disc • Free text boxes for further information.
Consent	• Type of consent for the postmortem including if consent was given for histological analysis and research of tissue.
Cause of death	• There are four tables within this form 1. Cause of death e.g. ascending infection 2. Abnormalities e.g. of the cord, placenta or organ 3. Pathologists opinion of the cause of death (free text) and separate entry area for cause of death assigned during analysis for this research 4. Quick classification system of those cases with Diabetes Mellitus/ obesity/ previous fetal loss.

Table 1 ***Details of new tables and forms entered into the database.***

2.2 Data Entry

Over a six month period, postmortem and antenatal details available for all stillbirth (>24 weeks), early (<20 weeks) and late (20-24 weeks) intrauterine deaths, dating from 2005 – 2013 (inclusive), from their paper records stored at Great Ormond Street Hospital, London were entered onto the database. (The term 'Miscarriage' will

be used throughout this thesis for ease of all pre-viable intrauterine deaths to distinguish them from stillbirth at 24 weeks of gestation and beyond). This gave a total of 817 cases. Despite Great Ormond Street Hospital being a tertiary referral centre for Paediatric deaths and complex anomalies, all stillbirth cases used in this study were referred from local District General Hospitals in the surrounding region as part of an existing service level agreements in which Great Ormond Street hospital was the regional referral centre for stillbirth autopsies. The data is therefore likely to be a valid representation of stillbirths one would expect from any other region in the UK since perinatal pathology services are regionalised. Data was collected and documented on second trimester intrauterine deaths (miscarriages) in addition to third trimester stillbirths to allow for comparison of associated findings between the different gestational ages at death and as a point of interest since minimal research has previously been completed on miscarriage autopsy findings compared to stillbirth. Further data was collected from St Georges Hospital, London to include 247 third trimester stillbirths (from the years 2012 and 2013) in order to increase the number of third trimester stillbirths within the study since stillbirths were not routinely examined at Great Ormond Street Hospital in the early years of the study. Due to the nature of the database, cases from the year 2012 from St Georges were coded on the database with a PM number of 21Pxxx and the 2013 cases as 31Pxxx.

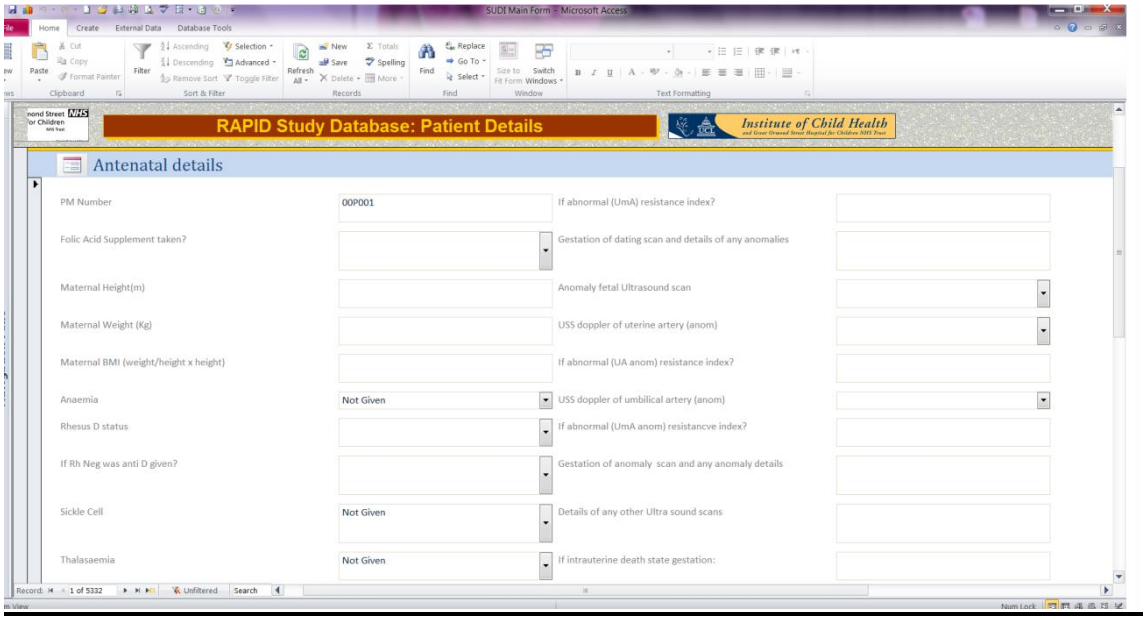

Figure 7 *Screen shot of the antenatal details form in the database*

The data entered for each case was obtained from the postmortem pack, which included the completed, typed autopsy report as authorised by a specialist Consultant Paediatric Pathologist, the completed consent form for the autopsy, a copy of the antenatal history and a completed perinatal death form to include the nature of the death (e.g. intrapartum or intrauterine) and surrounding circumstances. The completeness of the information in the postmortem pack was very variable and thus a potential limiting factor in this study. Not all cases contained maternal demographic information or a detailed review of the antenatal history. Any information provided was entered onto the database and in those cases without the information provided data fields were left blank or filled in with the appropriate dropdown option of "Not given". However, the aim of the current study was to determine the relative contribution of aspects of the autopsy as available to the pathologist at the time of the procedure, therefore the information provided is exactly comparable to routine clinical care. Data entry was standardised using two protocols; one designed previously for the original database and a second that was designed specifically for data entry into the new tables/forms (A*ppendix 2*) (184). Cases in

which the fetus received resuscitation immediately after delivery but did not have any signs of life were included in the study. Cases were excluded if the fetus gained or re-gained signs of life after resuscitation and in which an extremely premature fetus was delivered alive but survived without any intervention for a limited amount of time (usually less than 1 hour).

Once entered all the data was analysed through queries and statistical tests run through Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Any statistical test within this thesis can be viewed in detail in Appendix 3. Statistical tests used included, Mann Witney, Regression Analysis, Chi Squared and ROC Curves. Each of the results chapters contain a separate Methods sections which describe any additional methods used for specific analysis.

2.3 Histology and Laboratory Work

Histology and laboratory work account for a small part of the project and therefore the specific methods are discussed in the relevant Chapters (Chapters 9 and 10).

3. Population Demographics

3.0 Background

3.1 Methods

3.2Results

- 3.2.1 Total number of cases
- 3.2.2 Seasonal distribution of cases
- 3.2.3 Fetal Gender
- 3.2.4 Maternal Age
- 3.2.5 Maternal Ethnicity
- 3.2.6 Maternal Body Mass Index (BMI)
- 3.2.7 Maternal Smoking
- 3.2.8 Maternal Drugs and Alcohol
- 3.2.9 Previous Maternal obstetric history
- 3.2.10 Any relevant gynaecological history
- 3.2.11 IVF Pregnancies:
- 3.2.12: Diabetes Mellitus (IDDM, GDM)
- 3.2.13: Maternal Hypertension:

- 3.2.14 Maternal Infection
- 3.2.15 Multiple pregnancies

3.3 Discussion

3.0 Background

There are an estimated 1.02 million intrapartum stillbirths each year and a total of at least 3.2 million stillbirths worldwide (30). In 2013 there were 781,932 births in the United Kingdom of which 3,286 were stillborn, calculating a rate of 4.2 stillbirths per 1000 births (185).

Epidemiological associations include a parity of zero or greater than 3; mothers of African, African –Caribbean, Indian or Pakistani ethnicity and mothers with a body mass index over 30 (52, 186). Studies in London and Australia further highlight the role of ethnicity in stillbirth reporting that black and Asian women have twice the odds of stillbirth compared to white women even after adjusting for age, parity, deprivation, obesity, hypertension and diabetes and that mothers who were native to South Asia have an increased risk of antepartum stillbirth in late pregnancy (37 -42 weeks gestation) compared to other women in an Australian population (94, 95).

Other associations include smoking; maternal Diabetes Mellitus; history of antenatal vaginal bleeding and raised maternal age; women between the ages of 30-34 have a 27% greater risk of stillbirth (and other obstetric events) compared to mothers between the ages of 25-29, after adjusting for parity, ethnicity, body mass index, maternal co-morbidities and deprivation (52, 96). Women aged over 40 years also have an increased risk of stillbirth (97, 187).

In view of the many maternal demographic associations to stillbirths, this chapter will provide an overview of the demographics of this study's population (both maternal and fetal), to allow for comparison with national and published statistics.

3.1 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriage from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Data was analysed through queries and statistical tests run using Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Statistical tests can be viewed in detail in Appendix 3.

3.2 Results

3.2.1 Total population

Data was collected from 1,064 individual autopsy reports, from fetuses with intrauterine and intrapartum death delivered from 12 weeks gestation to term. *Table 2* shows the total number of cases in each death category; early miscarriage, late miscarriage and stillbirth.

	Early miscarriage	Late miscarriage	Stillbirth	Total
Total Number of cases	246 (23%)	179 (17%)	639 (60%)	1064

Table 2 Number of cases for each category of death in the overall study population

The majority of the cases are third trimester stillbirths. *Table 3* shows the distribution of all cases dependant on whether the death was intrauterine (more or less than 24 hours) or intrapartum (known or fresh) for the total number of cases. *Tables 4-6* show the distribution in the timing of death i.e. intrauterine or intrapartum, for each death category. In both the Early Miscarriage and Stillbirth categories the most common type of death was intrauterine with retention of the fetus for more than 24 hours in utero. In the late miscarriage group the most common type of death was a fresh intrapartum death.

	Intrauterine death < 24 hours	Intrauterine death > 24 hours	Intrapartum death – known	Intrapartum death – fresh	Total
Total Number of cases	138 (13%)	628 (59%)	29 (3%)	269 (25%)	1064

Table 3 Total cases split into different types of deaths

	Intrauterine death < 24 hours	Intrauterine death> 24 hours	Intrapartum death - known	Intrapartum death - fresh	Total
Early miscarriage	25 (10%)	127 (52%)	5 (2%)	89 (36%)	246

Table 4 Type of death in early miscarriage

	Intrauterine death < 24 hours	Intrauterine death> 24 hours	Intrapartum death - known	Intrapartum death - fresh	Total
Late miscarriage	21 (12%)	71 (39%)	6 (3%)	81 (45%)	179

Table 5 Type of death in Late miscarriage

	Intrauterine death < 24 hours	Intrauterine death> 24 hours	Intrapartum death - known	Intrapartum death - fresh	Total
Stillbirth	92 (14%)	430 (67%)	18 (29%)	99 (15%)	639

Table 6 Type of death in stillbirth

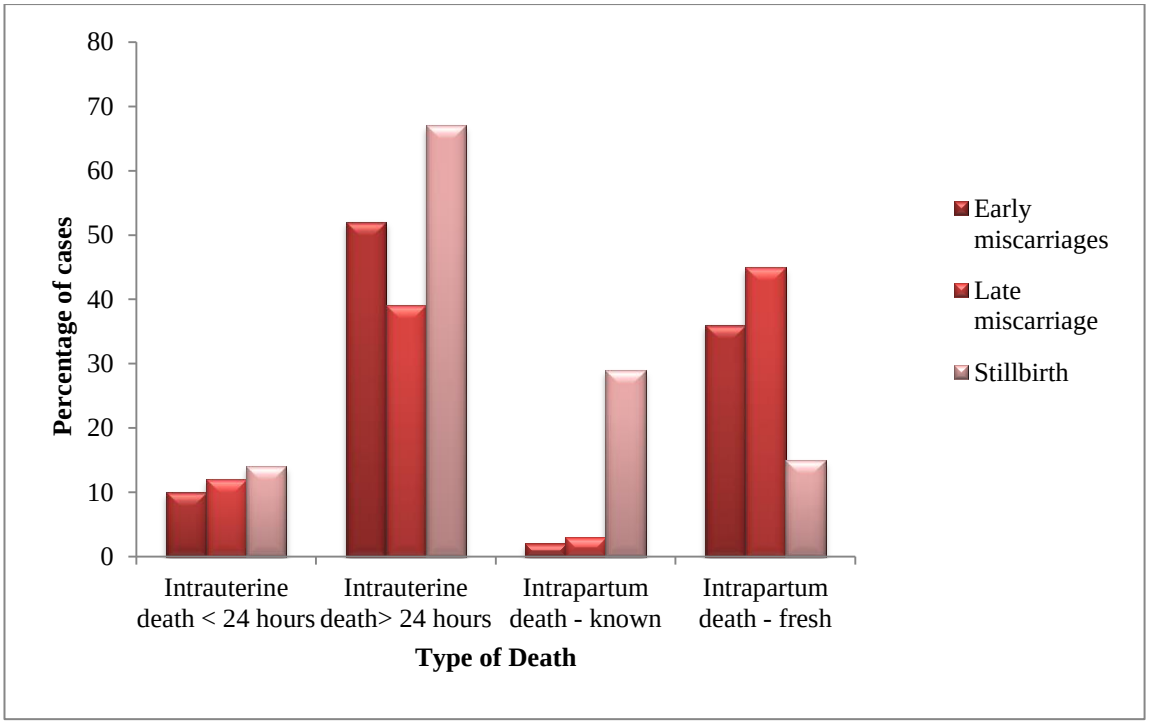

Figure 8 *The percentage of each death type within each death category*

3.2.2 Seasonal distribution of cases

Season	Total number of cases
Spring	239 (23%)
Summer	330 (31%)
Autumn	260 (25%)
Winter	229 (22%)
Totals:	**1058**
No information provided	6

Table 7 Seasonal distribution of cases

Season	Early Miscarriage	Late Miscarriage	Stillbirth
Spring	53 (22%)	46 (26%)	140 (22%)
Summer	75 (31%)	54 (30%)	201 (32%)
Autumn	64 (26%)	39 (22%)	157 (25%)
Winter	52 (21%)	40 (22%)	137 (22%)
Totals:	**244**	**179**	**635**
No information provided	2	0	4

Table 8 Seasonal distribution of cases within each death category

There was a non-significant peak in deliveries in the summer months.

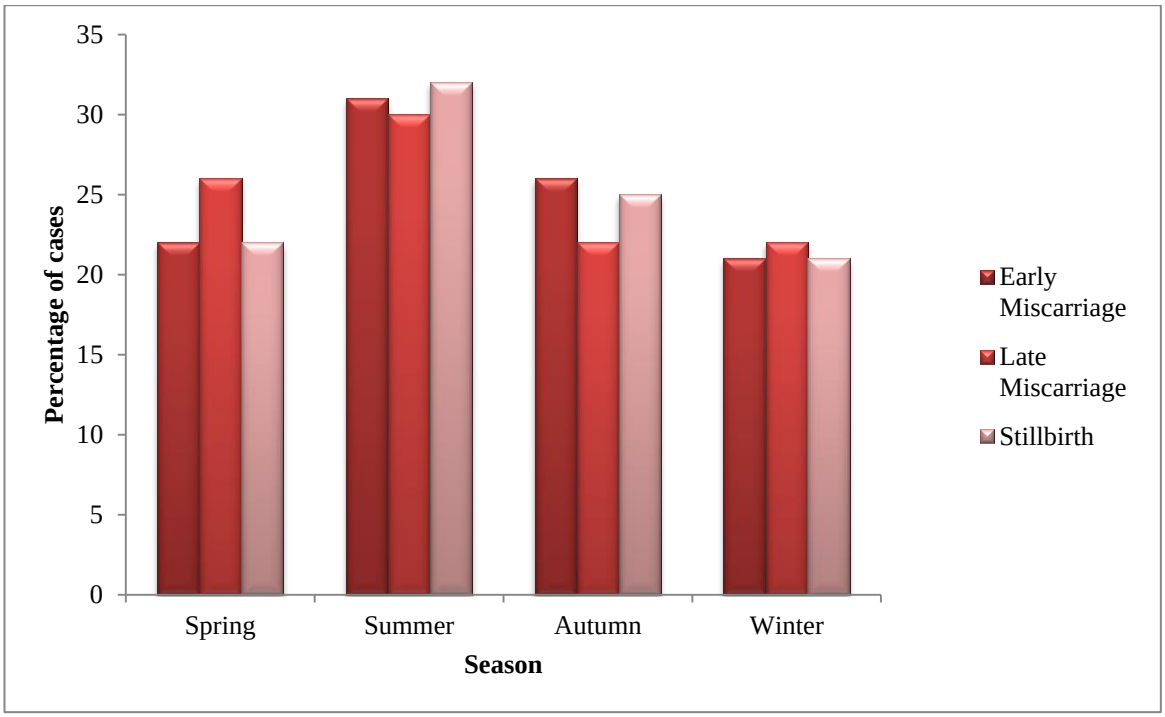

Figure 9 *Seasonal distribution of cases in each death category*

3.2.3 Fetal Gender

Fetal Gender	Number of cases
Male	569 (53%)
Female	466 (44%)
Unknown at PM / no information given	29 (3%)
Total:	1064

Table 9 Fetal gender within the total population studied.

Fetal Gender	Early miscarriage	Late miscarriage	Stillbirth
Male	129 (52%)	92 (51%)	348 (54%)
Female	101 (41%)	80 (45%)	285 (45%)
Unknown at PM / no imformation given	16 (7%)	7 (4%)	6 (1)
Total:	246	179	639

Table 10 Fetal Gender by death category

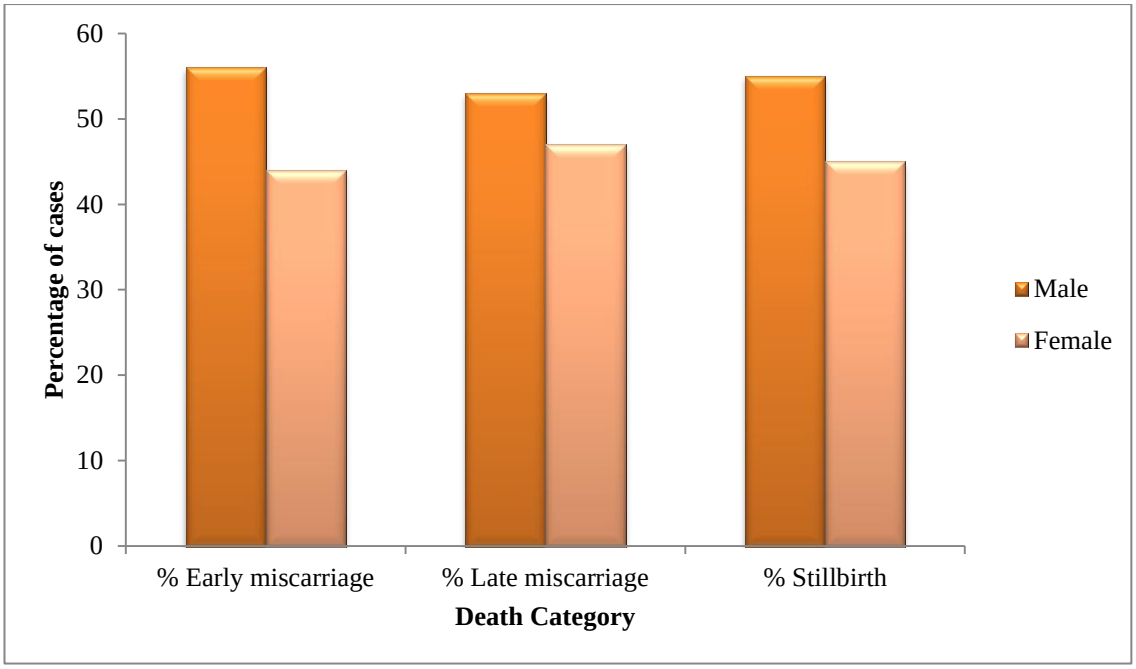

Figure 10 *Fetal Gender within each death category (excluding unknowns). There is a similar distribution of gender between categories of death but overall a greater number of male fetuses (not significant).*

There was no significant difference in the proportion of male to female fetuses between the miscarriage and the stillbirth categories (z = 0.0003, p = 0.10). There was also no significant difference between the observed and expected number of males and females in both miscarriage and stillbirth (z = 0.38, p = 0.70 and z=0.36, p=0.72 respectively).

3.2.4 Maternal Age

Maternal Age (years)	Study population
14 and under	1 (<1%)
15-19	43 (4%)
20-24	131 (13%)
25-29	241 (23%)
30-34	330 (32%)
35-39	209 (20%)
40-44	80 (8%)
45-49	4 (<1%)
50 and over	0
Total	1039
No Information given	25

Table 11 Maternal age in the study population

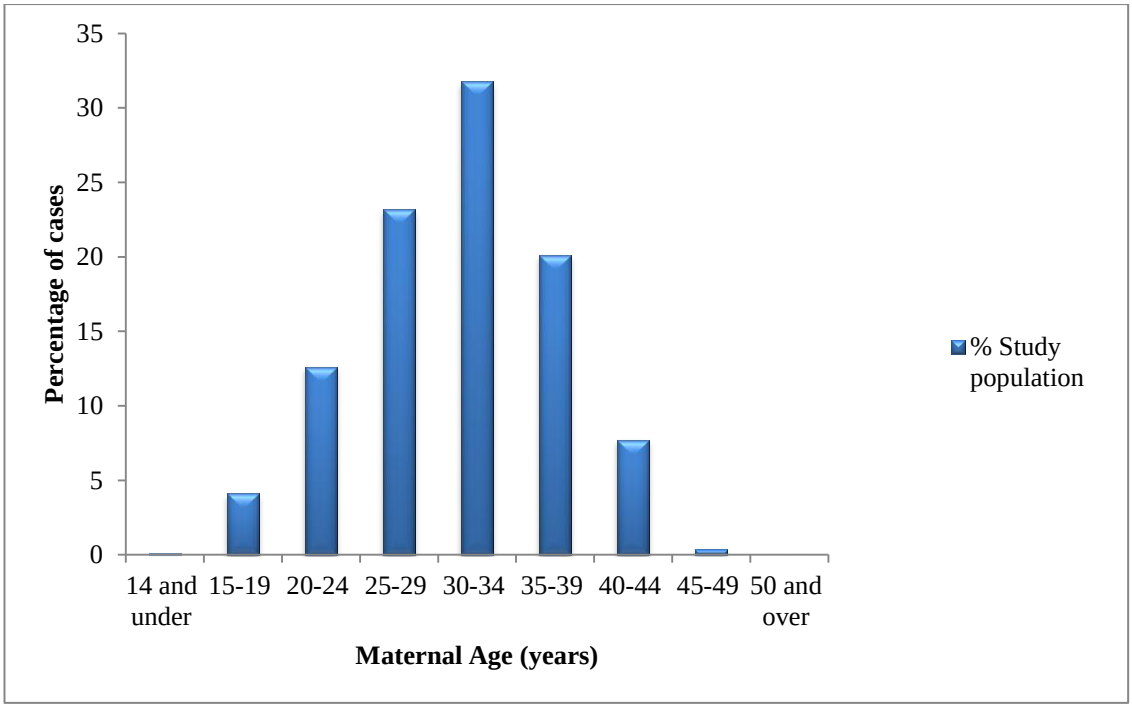

Figure 11 *Distribution of maternal age*

Maternal Age (years)	Early miscarriage	Late miscarriage	Stillbirth
14 and under	1(<1%)	0 (0%)	0 (0%)
15-19	7 (3%)	4 (2%)	32 (5%)
20-24	31 (13%)	20 (11%)	80 (13%)
25-29	47 (20%)	37 (21%)	157 (25%)
30-34	69 (29%)	66 (37%)	195 (31%)
35-39	62 (26%)	31 (18%)	116 (19%)
40-44	23 (10%)	17 (10%)	40 (6%)
45-49	0 (0%)	2 (1%)	2 (<1%)
50 and over	0 (0%)	0 (0%)	0(0%)
Total	**240**	**177**	**622**
Unknown	6	2	17

Table 12 Maternal age distribution by death category

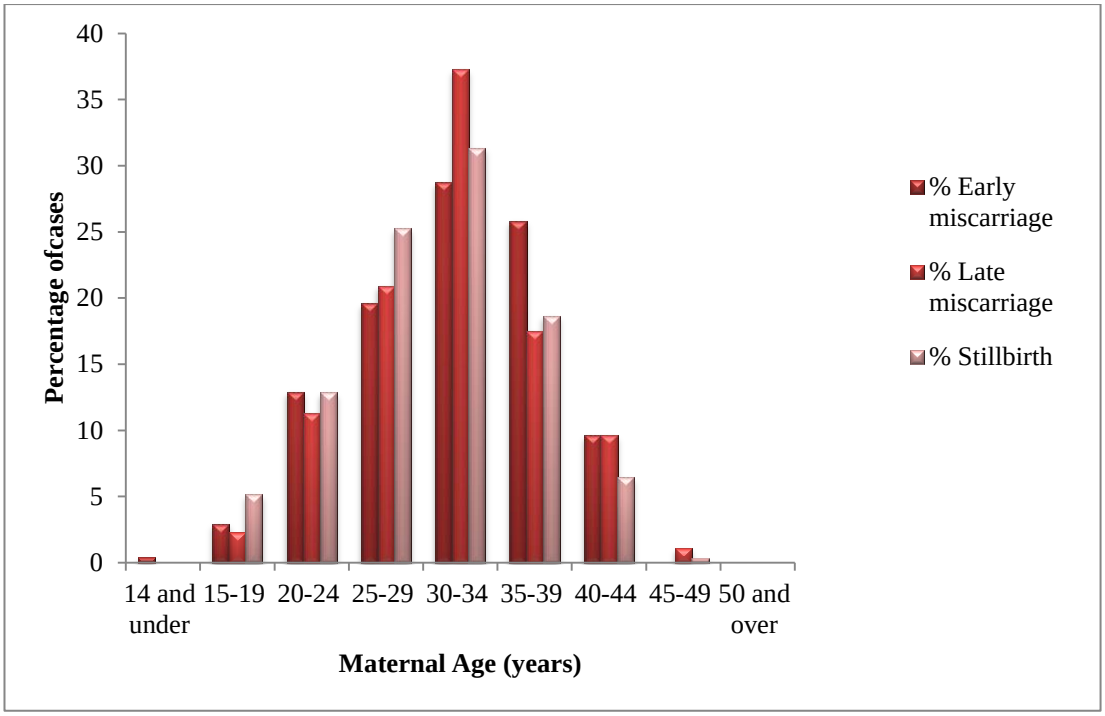

Figure 12 *Maternal age distribution by death category (excluding not givens)*

There is a peak of mothers in the age group 31-45 years who experience a fetal loss. The mean maternal age of the population studied is 31 years. There is no significant difference in the maternal age distribution between the stillbirth and miscarriage groups.

National information and data regarding the antenatal period, labour details and some postpartum details are collected by the Health and Social Care Information centre in the UK (188). For the purposes of this study data from the years 2012/2013 from this resource has been used as control data against the study population.

Data can be compared between the national distribution of maternal age (for all pregnancies) with the distribution of maternal age from this study's population as a whole (miscarriages and stillbirths).

Maternal Age	National Data 2012	Study population
14 and under	223 (<1%)	1 (<1%)
15-19	30,650 (5%)	43 (4%)
20-24	120,302 (18%)	131 (12%)
25-29	185,856 (28%)	241 (23%)
30-34	196,593 (29%)	330 (31%)
35-39	102,775 (15%)	209 (20%)
40-44	24,342 (4%)	80 (8%)
45-49	1,343 (<1%)	4 (<1%)
50 and over	114 (<1%)	0 (<1%)
unknown	9,057 (1%)	25 (2%)
Total:	**671,255**	**1064**

Table 13 Maternal age distribution for national data and study population

There is a significant difference in the distribution of the maternal ages of the two populations ($p = 0.0012$) with women within the study being significantly older than the general population of women in all pregnancies nationally. (*Table 13*, *Figure 13).*

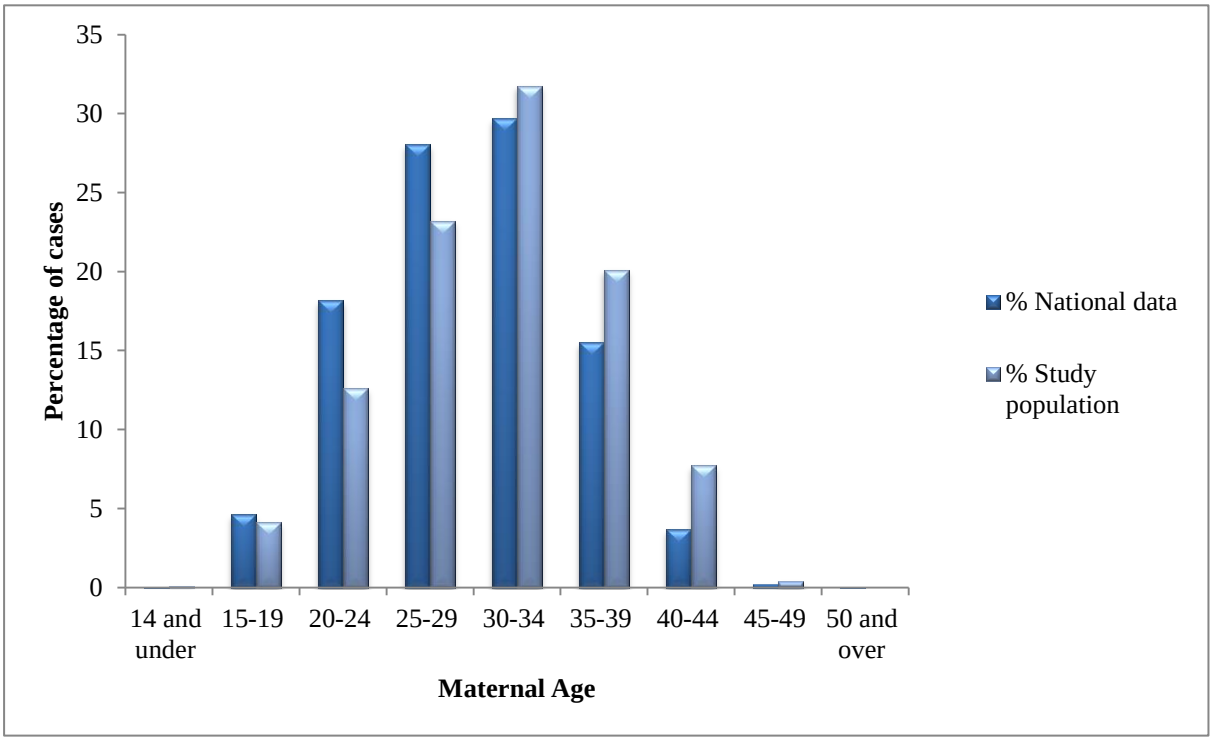

Figure 13 *Distribution of maternal age, national data and study population*

The two populations can be compared further, looking at the distribution of maternal age for both stillbirths and miscarriages separately, between the national data and the study population.

When comparing the national data for stillbirths with the study's stillbirth population (see table 14) there is no significant difference in the maternal age distribution (p= 0.16). However compared to the overall national maternal age distribution the study's stillbirth population is significantly older (p = 0.0003). These data demonstrate that women suffering intrauterine death are, on average, older than the overall maternity population but importantly demonstrate that the current study population is representative of the national population of women suffering stillbirth.

Maternal Age	National data – stillbirths	Study population - stillbirths
14 and under	0 (0%)	0 (0%)
15-19	183 (6%)	32 (5%)
20-24	614 (19%)	80 (13%)
25-29	882 (27%)	157 (25%)
30-34	873 (27%)	195 (31%)
35-39	476 (15%)	116 (18%)
40-44	147 (5%)	40 (6%)
45-49	13 (0%)	2 (0%)
50 and over	1 (0%)	0(0%)
No Information	31 (31%)	17 (3%)
Total:	**3,220**	**639**

Table 14 Maternal age distribution for both the national data and the study population of stillbirths.

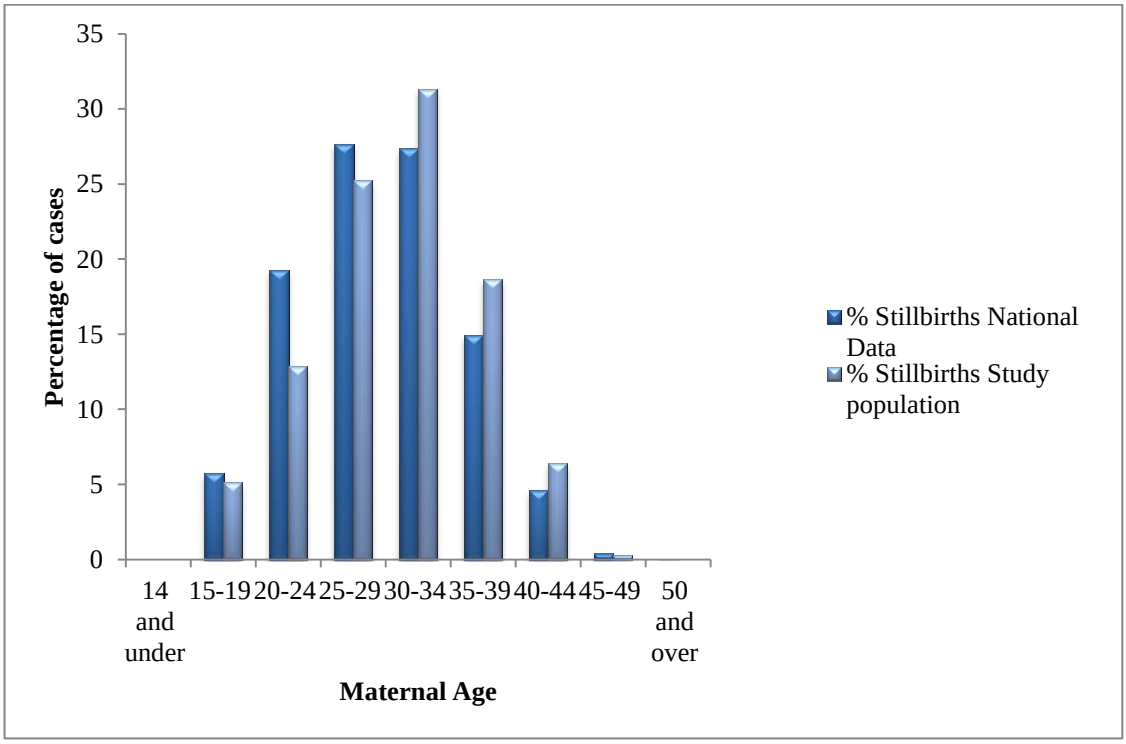

Figure 14 *Maternal age distribution for stillbirths in the national data and study population (Excluding unknowns)*

When comparing the national data for maternal age in those with miscarriages against the study's population of miscarriages (early and late) there was a significant difference in the distribution of maternal age ($p= 0.0008$). The study's population is significantly older than the national population of women who had miscarriages and significantly older than all pregnant mothers ($p<0.0001$).

Maternal Age	National data - miscarriages	Study population - miscarriages
14 and under	34 (0%)	1 (<1%)
15-19	1959 (5%)	11 (3%)
20-24	5842 (15%)	51 (12%)
25-29	8589 (22%)	84 (21%)
30-34	9809 (25%)	135 (32%)
35-39	7635 (19%)	93 (21%)
40-44	3845 (10%)	40 (9%)
45-49	366 (1%)	2 (<1 %)
50 and over	609 (2%)	0 (<1 %)
Unknown	1112 (3%)	8 (<1%)
Totals:	**39800**	**425**

Table 15 Distribution of maternal age for national data and study population

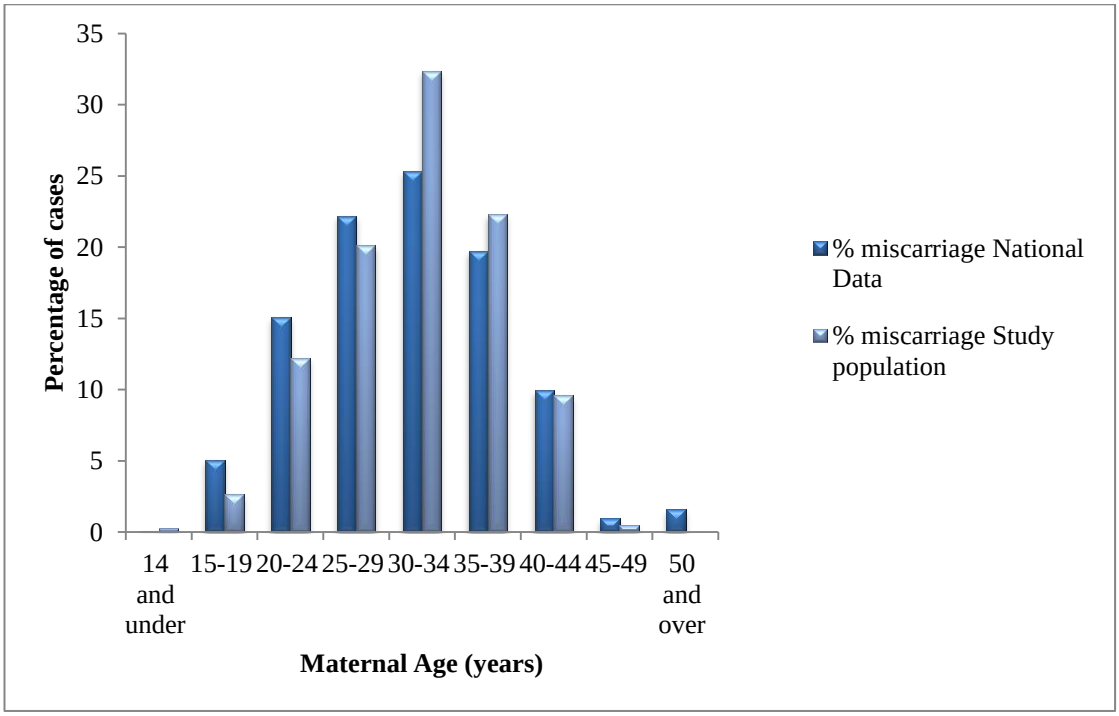

Figure 15 *Distribution of maternal age for both the national data for miscarriage and study population of miscarriage (Excluding unknowns)*

3.2.5 Maternal Ethnicity

Ethnicity	Number of cases (Study population)
Not Given	313 (29%)
White, British	276 (26%)
White, Irish	7 (1%)
White, Other/NOS	186 (17%)
Mixed, White and Black Caribbean	9 (1%)
Mixed, White and Black African	1 (<1%)
Mixed, White and Asian	0 (<1%)
Mixed, Other/NOS	5 (<1%)
Asian/Asian British, Indian	21 (2%)
Asian/Asian British, Pakistani	6 (1%)
Asian/Asian British, Bangladeshi	2 (<1%)
Asian/Asian British, Other/NOS	29 (3%)
Black/Black British,Caribbean	43 (4%)
Black/Black British, African	149 (14%)
Black/Black British, Other/NOS	14 (1%)
Chinese	3 (<1%)
Total:	**1064**

Table 16 Maternal ethnicity in the study population

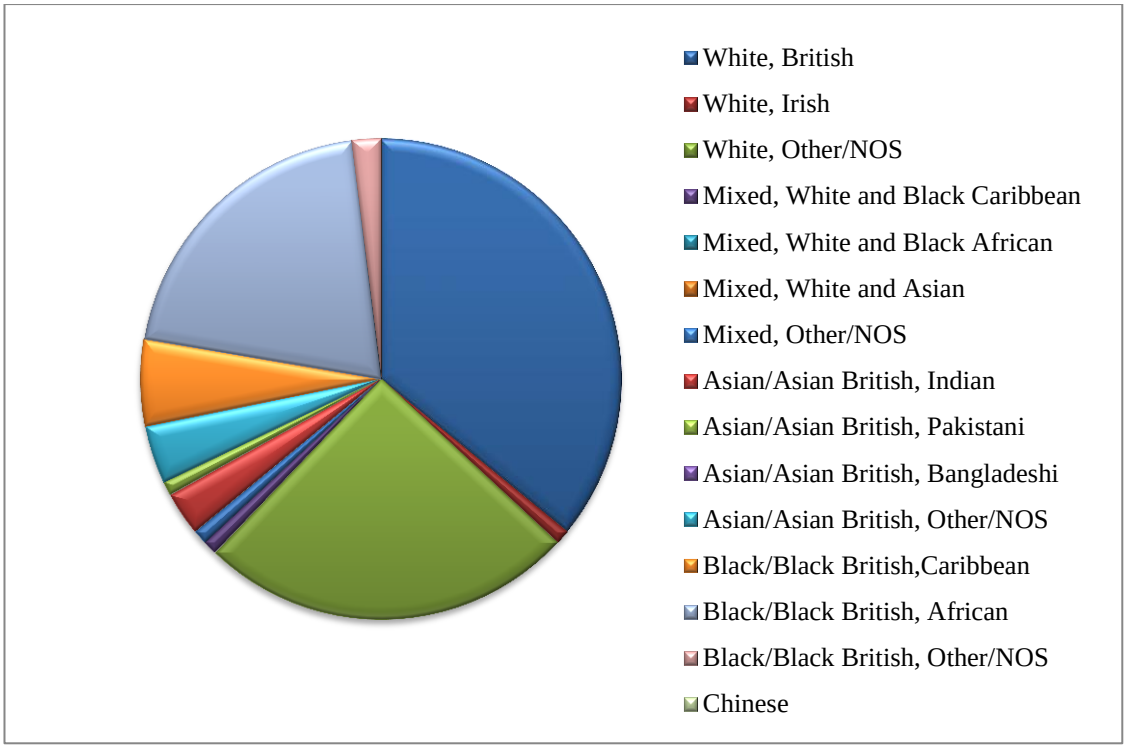

Figure 16 *Maternal ethnicity in the study population (excluding unknowns)*

The ethnic groups are simplified below into White, Asian, Black, Oriental/other and Mixed (excluding not givens) and each death category. (*Table 17*)

Maternal Ethnicity	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
White	87 (54%)	71 (53%)	311 (68%)	**469 (62%)**
Asian	14 (9%)	10 (7%)	34 (7%)	**58 (8%)**
Black	55 (34%)	52 (39%)	99 (22%)	**206 (27%)**
Oriental/Other	2 (1%)	0 (0%)	1 90%)	**3 (0%)**
Mixed	3 (2%)	2 (1%)	10 (2%)	**15 (2%)**
Total:	**161**	**135**	**455**	**751**

Table 17 Maternal ethnicity by death category (Excluding not givens)

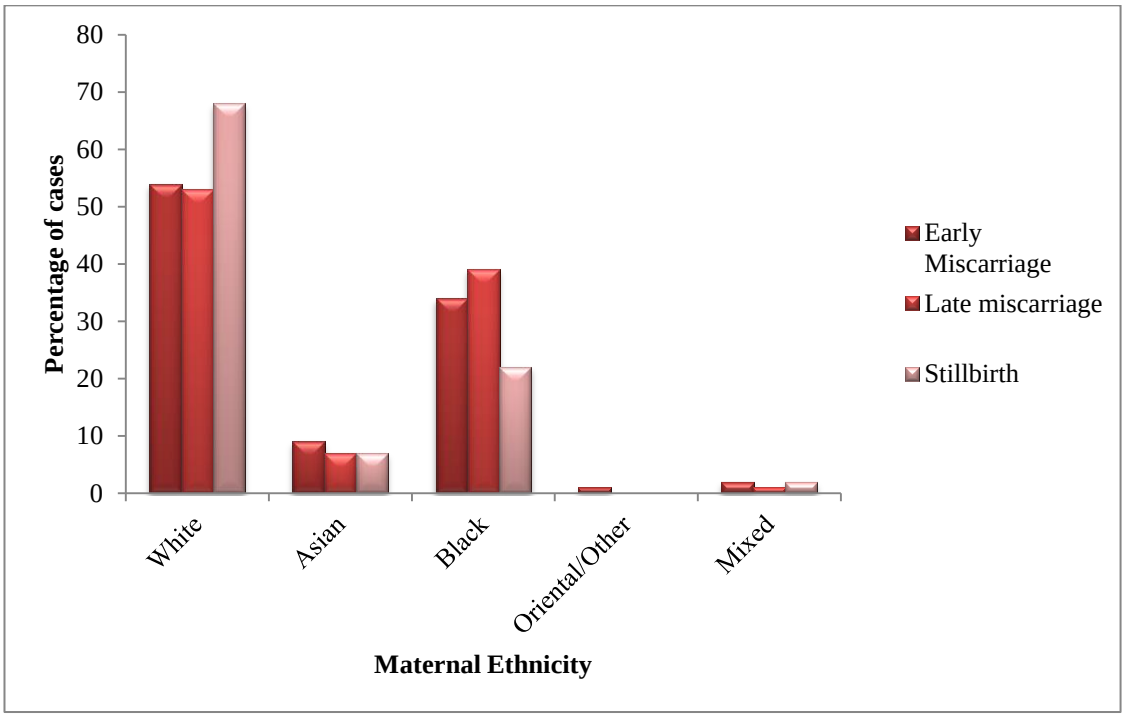

Figure 17 *Maternal ethnicity for each death category (excluding not givens)*

For each death category the largest proportions of death, as displayed in Figure 17 are to the white mothers, followed by black mothers. Statistically, when comparing miscarriages to stillbirths:

- White mothers are significantly overrepresented in the stillbirths compared to the miscarriages group compared to non-white mother ($z= 4.08$, $p <0.0001$).
- Black mothers are significantly overrepresented in the miscarriages compared to stillbirth groups when compared to non-black mothers ($z= 3.94$, $p <0.0001$).
- White mothers have a significantly greater proportion of stillbirths than black mothers ($z= 4.47$, $p <0.0001$)
- Black mothers have a significantly greater proportion of miscarriages than white mothers ($z= 4.47$, $p <0.0001$)
- There is no significance between mothers who are Asian versus Non- Asian and mothers of Mixed Ethnicity versus other ethnicity.

Using the national HSCIC data (188) as controls, a comparison can be made between the proportion of cases of each ethnicity (*Table 18*, *Figure 18).*

Maternal Ethnicity	National data	Study population
Asian	71,131 (11%)	58 (5%)
Black	33,105 (5%)	206 (19%)
Chinese / Other	24,204 (4%)	3 (< 1%)
White	482,095 (72%)	469 (44%)
Mixed	10,091 (2%)	15 (1%)
Not Stated	39,453 (6%)	0 (0%)
Unknown	11,176 (2%)	313 (29%)
Total:	**671,255**	**1064**

Table 18 Maternal ethnicity for both the national data and the study population.

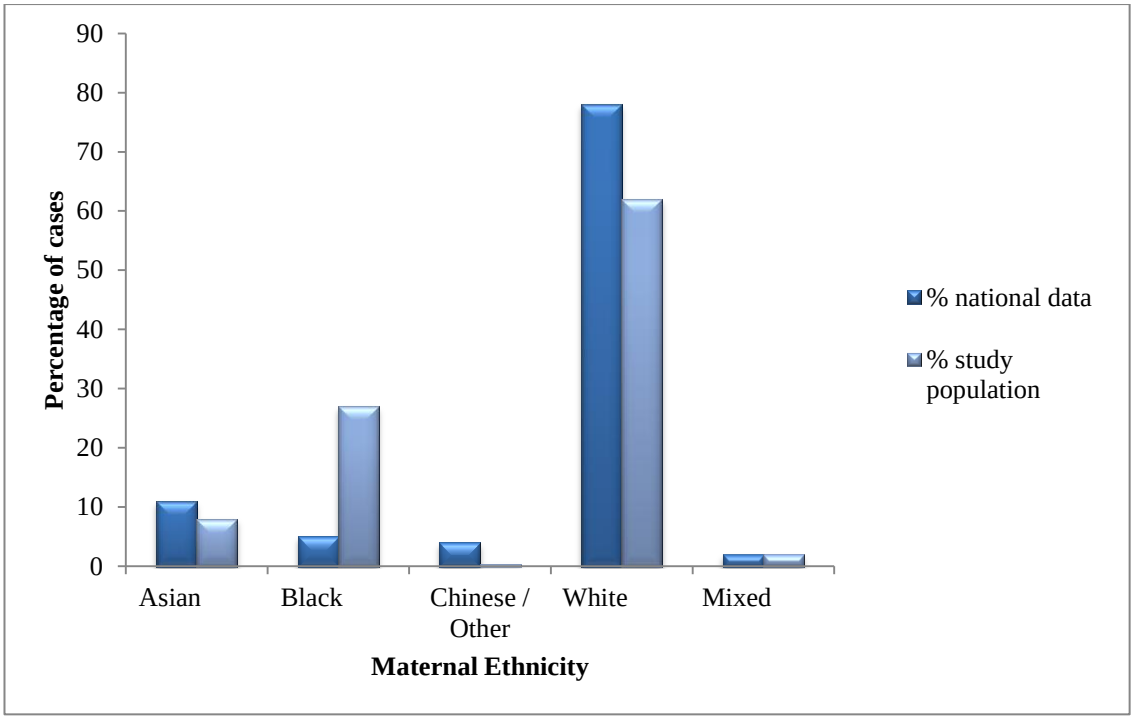

Figure 18 *Maternal ethnicity within the national data and the study population. (Excluding unknowns)*

There is greater proportion of unknown maternal ethnicities in the study population but overall the most represented ethnic group is white.

Black mothers, are significantly overrepresented in the study population ($z = 28.06$, $p < 0.0001$).

3.2.6 Maternal Body Mass Index (BMI)

The body mass index of the mothers was defined as:

- BMI underweight= <18
- BMI normal = 18- 24
- BMI overweight = 25-29
- BMI obese = >30 (189)

Body Mass Index (BMI)	Number / percentage of cases
BMI underweight	5 (<1%)
BMI normal	152 (14%)
BMI overweight	167 (16%)
BMI obese	143 (13%)
BMI not given	597 (56%)
Total:	**1064**

Table 19 Maternal BMI in study population

Body Mass Index (BMI)	Early miscarriage	Late miscarriage	Stillbirth
BMI underweight	1 (<1%)	0 (0%)	4 (1%)
BMI normal	42 (17%)	25 (14%)	85 (13%)
BMI overweight	43 (17%)	34 (19%)	90 (14%)
BMI obese	41 (17%)	20 (11%)	82 (13%)
BMI not given	119 (48%)	100 (56%)	378 (59%)
Totals:	**246**	**179**	**639**

Table 20 Maternal BMI within each death category

Unfortunately more than half of the study's population had no reported maternal Body Mass Index (BMI) in their antenatal notes or history as shown in *Table 20, Figure 19* represents the same data with the exclusion of the unknown BMIs. In both the early miscarriage and stillbirth groups the majority of the study's population of mothers had normal BMIs. For Late miscarriage there was a slightly higher proportion of overweight mothers and no underweight mothers.

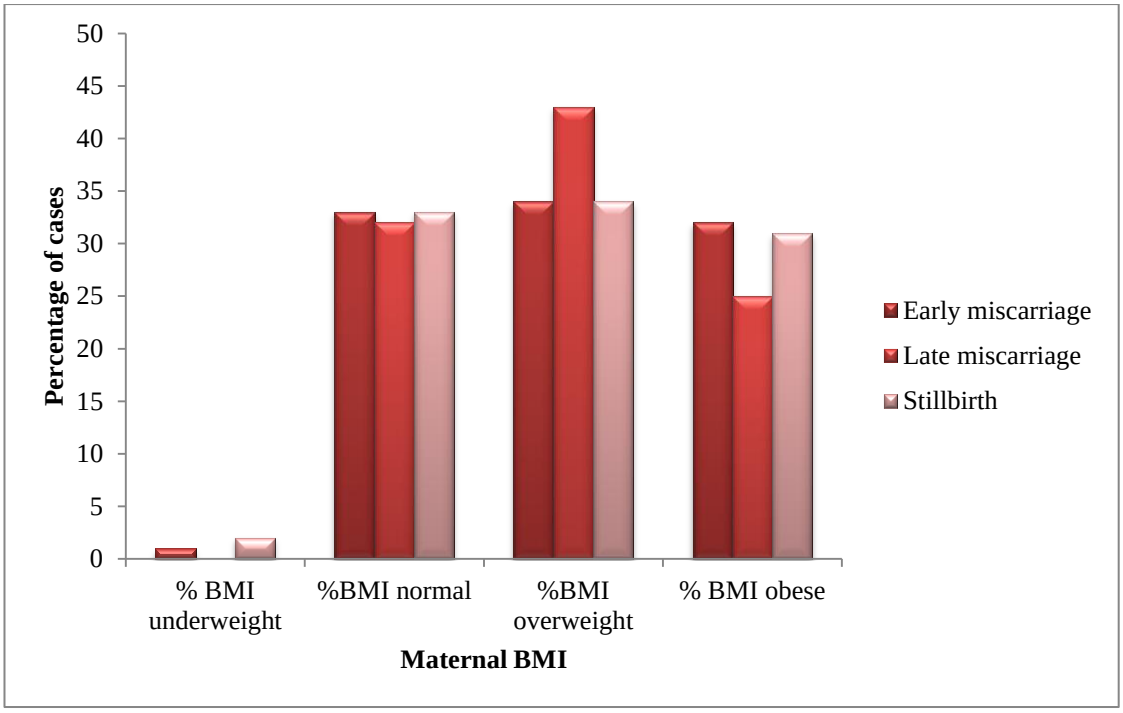

Figure 19 *Maternal BMI within each death category.*

There are no significant differences between the miscarriage and stillbirth groups when comparing body mass index.

Using national data from the Health Survey for England 2012, HSCIC (190) as controls, the study population is significantly more overweight (z= 2.996, p = 0.0027) and more obese (z= 3.920, p <0.0001) than the total population of women of childbearing age confirming obesity is a risk factor for fetal loss (*Table 21* and *Figure20*).

(National figures for obesity had to be taken from women of a child bearing age between 25- 34 years from the year 2012 as no data is currently available covering the body mass index of all pregnant mothers in the United Kingdom).

Maternal Body Mass Index	National data for all women aged 25 - 34 in 2012	Study population
Underweight	11 (2%)	5 (1%)
Normal	275 (49.9%)	152 (33%)
Overweight	149 (27%)	167 (36%)
Obese	116 (21.2%)	143 (31%)
Total	**551**	**467**

Table 21 Maternal BMI in the study population and the national population of women (between the ages of 25-34 years in 2012)

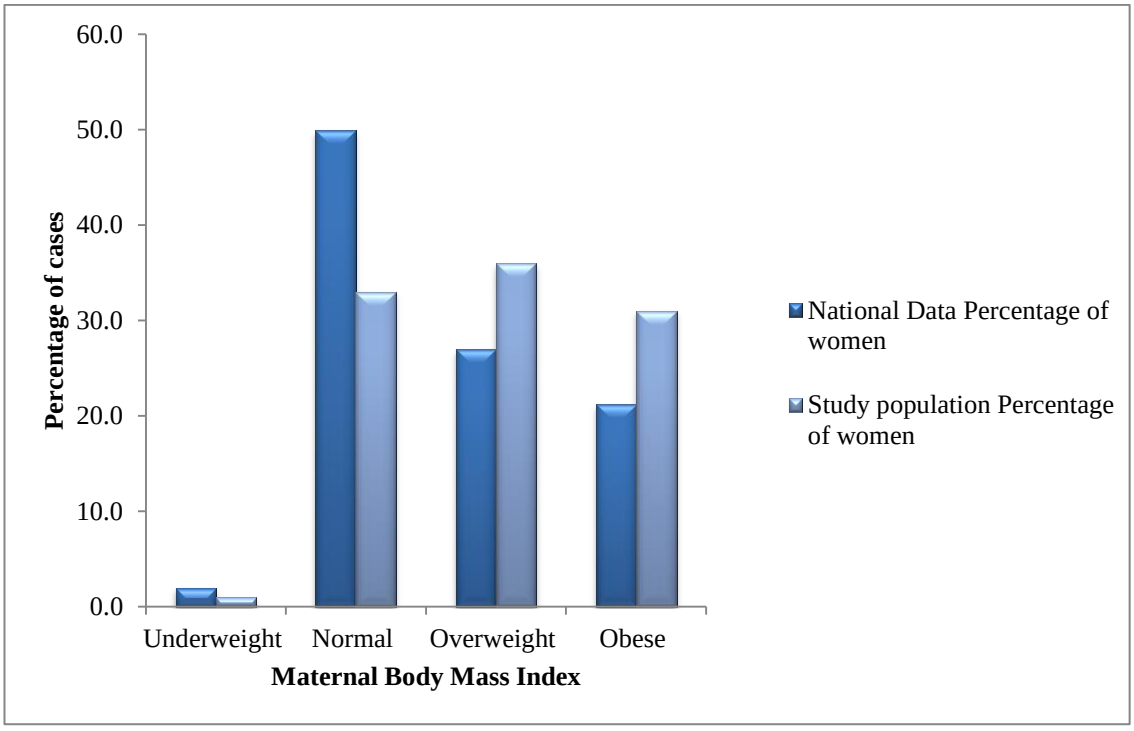

Figure 20 Maternal BMI in the study population and the national population of women (between the ages of 25-34 years in 2012)

These data confirm that maternal overweight and obesity is associated with increased risk for felt loss, but interestingly this risk appears similar for intrauterine death at all gestational ages and is not restricted to late third trimester stillbirth.

3.2.7 Maternal Smoking

Maternal smoking status	Total number of cases
Not given	364
Smoker	111(16%)
Non-smoker	589 (84%)
Total:	**1064**

Table 22 Maternal smoking status during pregnancy (percentages exclude not givens)

Maternal smoking status	Early Miscarriage	Late Miscarriage	Stillbirth
Not given	96	61	207
Yes smoker	18 (12%)	17(14%)	76 (18%)
Non-smoker	132 (88%)	101 (86)	356 (82%)
Total:	**246**	**179**	**639**

Table 23 Maternal smoking status by death category during pregnancy (percentages exclude not givens)

The majority of the mothers in the study population were non-smokers but of those that did smoke, the majority had stillbirths rather than miscarriages. No further analysis of these cases has been performed due to the small numbers and potential issues relating to self- reporting of use.

3.2.8 Maternal Drugs and Alcohol

Maternal illicit drug use	Total Number of cases
Not given	594
Illicit Drugs	17 (4%)
No Illicit drugs	453 (96%)
Total:	**1064**

Table 24 Maternal illicit drug use during pregnancy (percentages exclude not givens)

Maternal Illicit drug use	Early miscarriage	Late miscarriage	Stillbirth
Not given	168	100	326
Illicit Drugs	2 (3%)	2 (3%)	13 (4%)
No illicit drugs	76 (97%)	77 (37%)	300 (96%)
Total:	**246**	**179**	**639**

Table 25 Maternal illicit drug use by death category during pregnancy (percentages exclude not givens)

Maternal alcohol use	Total number of cases
Not given	492
Drank Alcohol	32 (6%)
No Alcohol	540 (94%)
Total:	**1064**

Table 26 Maternal alcohol use during pregnancy (percentages exclude not givens)

Maternal Alcohol use	Early miscarriage	Late miscarriage	Stillbirth
Not given	125	81	286
Drank alcohol	6 (5%)	8 (8%)	18 (5%)
No alcohol	115(95%)	90 (92%)	335 (95%)
Total:	**246**	**179**	**639**

Table 27 Maternal alcohol use, by death category during pregnancy (Percentages exclude not givens)

A very small proportion of mothers drank alcohol or took illicit drugs within our population. No further analysis of these cases has been completed.

3.2.9 Previous Maternal obstetric history

Past Maternal Obstetric History	Total number of cases
Primigravida	356 (68%)
G1+P0	168 (32%)
Total:	**524**

Table 28 Mothers with a past obstetric history of being either primigravida or having one or more pregnancies but no live births (G1+P0)

One third (356/1064) of the mothers in the total study population were primigravida and a smaller proportion (16%) had experienced at least one previous pregnancy but had no live births. Their losses included miscarriages, stillbirths, terminations and ectopic pregnancies.

Previous Maternal obstetric history	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Primigravida	55 (56%)	54 (60%)	247(74%)	**356**
G1+P0	44 (44%)	36 (40%)	88 (26%)	**168**
Total:	**99**	**90**	**335**	**524**

Table 29 Mothers with a past obstetric history of being either primigravida or having one or more pregnancies but no live births (G1+P0) in relation to the type of death experienced in most recent pregnancy.

When comparing miscarriages to stillbirths:

- Mothers had a significantly greater proportion of stillbirths than miscarriages if they were primigravida, ($z = 4.38$, $p < 0.0001$) compared to mothers who were not primigravida.
- Mothers had a significantly greater proportion of miscarriages if they were G1+P0 ($z = 3.78$, $p < 0.0002$) compared to those mothers who were primigravida.

3.2.10 Relevant gynaecological history

Maternal Gynae History	Number of cases
History of Maternal fibroids	45 (4%)
History of Bicornate uterus	7 (1%)
History of Polycystic ovaries	18 (2%)
PV bleeding(this pregnancy)	70 (7%)
Previous cervical History	11 (1%)
IVF pregnancy (this pregnancy)	39 (4%)
Other gynae history	14 (1%)
No gynae history/ no history given	860 (81%)
Total:	**1064**

Table 30 Mothers with relevant gynaecology history.

A small proportion of the total number of cases had relevant gynaecological history. However, certain gynaecological features were associated with differences in presentation group. Specifically:

- Mothers with fibroids were significantly overrepresented in the miscarriage group compared to the stillbirth group ($z = 3.74$, $p = 0.0002$).
- Mothers with a history of PV bleeding during the pregnancy were significantly overrepresented in the miscarriage group compared to the stillbirth group ($z= 3.55$ $p =0.0004$)
- IVF pregnancies were significantly overrepresented in the miscarriage group compared to the stillbirth group ($z=3.81$, $p = 0.0001$).

Maternal Gynae History	Early miscarriage	Late miscarriage	Stillbirth
History of Maternal fibroids	16 (7%)	14 (8%)	15 (2%)
History of Bicornate uterus	1 (<1%)	2 (1%)	4 (1%)
H/O Polycystic ovaries	1 (<1%)	7 (4%)	10 (2%)
PV bleeding(this pregnancy)	26 (11%)	16 (9%)	28 (4%)
Previous cervical History	3 (1%)	3 (2%)	5 (1%)
IVF pregnancy (this pregnancy)	16 (7%)	11 (6%)	12 (2%)
Other gynae history	7 (3%)	2 (1%)	5 (1%)
No gynae history/ no history given	192 (78%)	124 (69%)	560 (88%)
Total:	**246**	**179**	**639**

Table 31 Mothers with relevant gynaecological histories in each death category

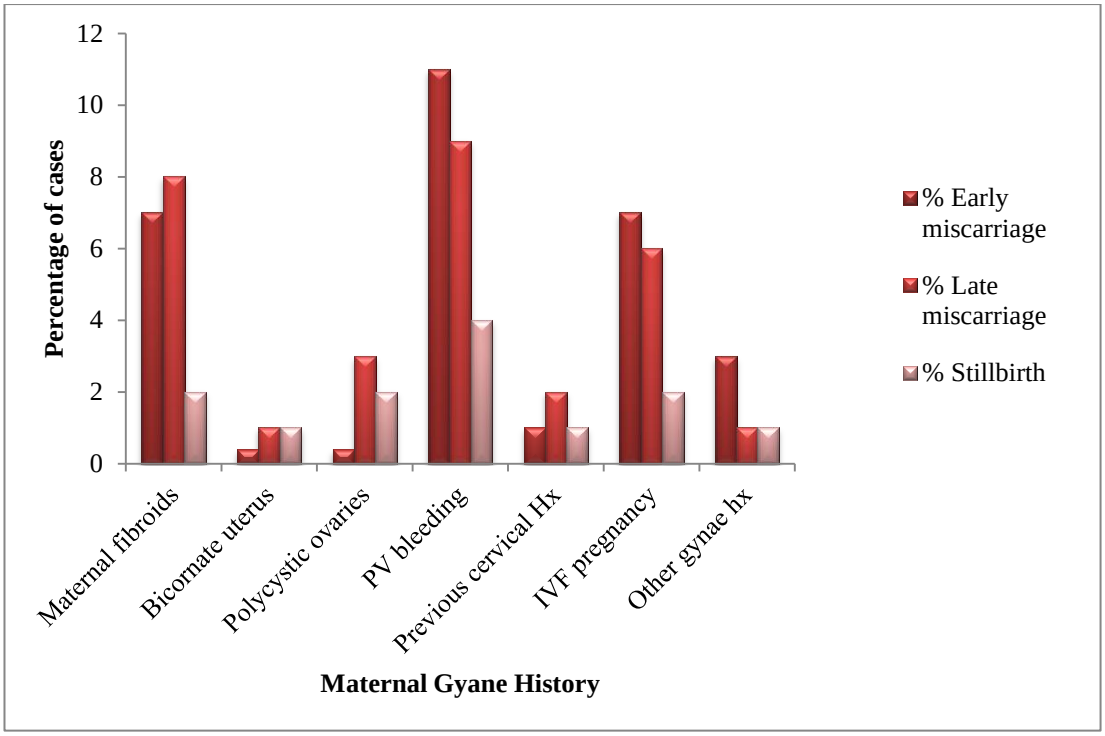

Figure 21 *Mothers with relevant gynaecological histories in each death category.*

3.2.11 IVF Pregnancies

	In total	Early Miscarriage	Late Miscarriage	Stillbirth
IVF Pregnancies	39	16 (41%)	11 (28%)	12 (31%)

Table 32 IVF pregnancies for each death category

A greater proportion of IVF pregnancies ended in miscarriage than stillbirth.

3.2.12 Diabetes Mellitus (IDDM, GDM)

DM – Diabetes Mellitus

IDDM – Insulin Dependent Diabetes Mellitus

GDM – Gestational Diabetes Mellitus

Maternal Diabetic History	Total Number of cases in study population
Not information given	**19 (2%)**
No DM	**982 (92%)**
Known DM	**26 (2%)**
• IDDM	18
• Non-IDDM	8
GDM	**37 (3%)**
• Non-insulin dependent GDM	14
• Insulin – dependent GDM	6
• GDM no info on meds given	17
Total:	**1064**

Table 33 Mothers with diabetes mellitus in the study population

Maternal Diabetic History	Early miscarriage	Late miscarriage	Stillbirth
No Information given	6	3	10
No DM	**231 (96%)**	**169 (96%)**	**582 (92%)**
Known DM	**3 (1)**	**4 (2%)**	**19 (3%)**
• IDDM	2	3	13
• Non-IDDM	1	1	6
Gestational DM	**6 (3%)**	**3 (2%)**	**28 (5%)**
• Non-Insulin dependent GDM	3	2	9
• Insulin dependent GDM	1	1	4
• GDM no info given on meds	2	0	15
Total:	**240**	**176**	**629**

Table 34 Mothers within each death category with Diabetes Mellitus/ Gestational Diabetes.

When comparing the number of women who had any form of diabetes in pregnancy against those who did not, stillbirths were significantly overrepresented compared to miscarriages ($z=2.41$, $p=0.02$).

There were no statistical differences between stillbirth and miscarriages groups when comparing any form of diabetes mellitus with any form of gestational diabetes mellitus.

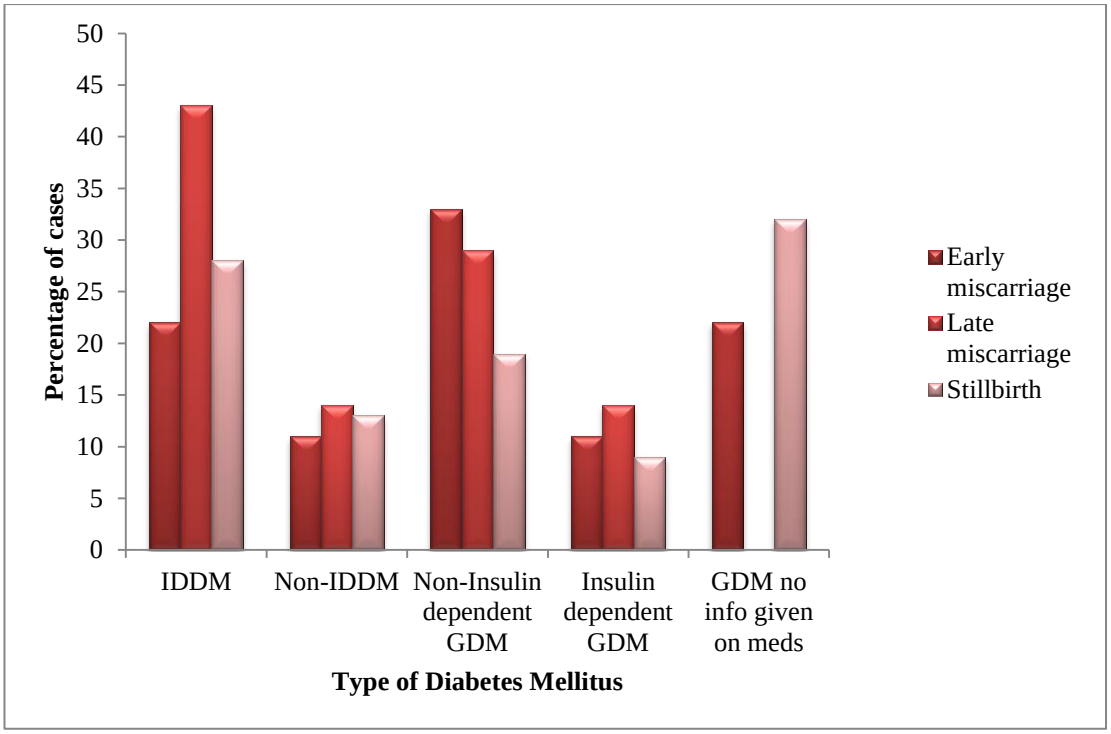

Figure 22 *Diabetes Mellitus in different death categories*

When comparing the study data against the national data for diabetes mellitus the study population had a greater proportion of mothers with some form of diabetes mellitus (odds ratio = 1.4).

Population	Number of cases of Diabetes Mellitus during Pregnancy	Total number of pregnancies
National data	28686 (4%)	671255
Study population (incl. DM and GDM)	63 (6%)	1064

Table 35 Diabetes mellitus during pregnancy in the study population and the national data

3.2.13 Maternal Hypertension

Blood Pressure (BP)	Total Number of cases
Not information given	**19 (2%)**
Normal BP	**959 (90%)**
Hypertension	**86 (8%)**
• Chronic	25
• Pregnancy induced	35
• Pre-eclampsia	26
Total:	**1064**

Table 36 Blood pressure findings in the study population

Blood Pressure	Early miscarriage	Late miscarriage	Stillbirth
Not given	**2 (1%)**	**0 (0%)**	**17 (3%)**
Normal BP	**236 (96%)**	**170 (94%)**	**553 (87%)**
Hypertension	**8 (3%)**	**9 (5%)**	**69 (11%)**
• Chronic	2	4	19
• Pregnancy induced	6	4	25
• Pre-eclampsia	0	1	25
Total	**246**	**179**	**639**

Table 37 Maternal blood pressure findings within each death category.

- Women with hypertension were significantly overrepresented in the stillbirths compared to the miscarriage groups ($z= 4.09$, $p <0.0001$).
- There was no difference in the proportions of mothers with chronic or pregnancy induced hypertension.
- Women suffering from pre-eclampsia were significantly overrepresented in the stillbirths compared to the miscarriage groups (in comparison to both chronic hypertension and pregnancy induced hypertension; $p =0.050$ and $p =0.017$ respectively).

When comparing the study data with national data on hypertension in pregnancy, no significant difference was found in the proportion of women in the study population

with hypertension (excluding pre-eclampsia and eclampsia) compared to those in the general pregnant population (z= 0.94 p = 0.35).

	Number of cases of hypertension (excluding eclampsia)	Total number of pregnancies
National Data	34244 (5%)	671255
Study population	60 (6%)	1045

Table 38 Any form of maternal hypertension in pregnancy (excluding pre-eclampsia) in the study population and the national data

3.2.14 Maternal Infection

Maternal Infection	Total number of cases in our population
No infection	957 (90%)
Other	23 (2%)
UTI	43 (4%)
Vaginal Infection	22 (2%)
HIV Positive	10 (1%)
Syphilis	4 (<1%)
Hep B Positive	5 (<1%)
Total:	**1064**

Table 39 Maternal infections within study population

Maternal Infection	Early Miscarriage	Late Miscarriage	Stillbirth
No infection	222 (90%)	152 (85%)	583 (91%)
Other	2 (1%)	5 (3%)	16 (3%)
UTI	15 (2%)	10 (6%)	18 (3%)
Vaginal Infection	4(2%)	5 (3%)	13 (2%)
HIV Positive	2 (1%)	4 (2%)	4 (1%)
Syphilis	0 (0%)	1 (1%)	3 (<1%)
Hep B Positive	1 (<1%)	2 (1%)	2 (<1%)
Total:	**246**	**179**	**639**

Table 40 Maternal infections within each death category

The majority of mothers in the study did not have any infection during pregnancy. The most common type of infection, when present, was urinary tract. No significant difference in the proportion of infected mothers was found between the miscarriage and stillbirth groups ($z = 1.71$, $p = 0.09$).

3.2.15 Multiple pregnancies

50 individual fetal deaths were from twin pregnancies; 3 fetuses were from triplet pregnancies (from 2 cases) (53 deaths in total out of 1064; 5%).

	Early Miscarriage	Late Miscarriage	Stillbirth	Total number of individual twin losses
Number of individual twin losses	9 (17%)	15 (28%)	29 (55%)	53

Table 41 The number and percentage of individual twin losses for each death category

There is currently no usable data on the rate of individual intrauterine/intrapartum fetal deaths in twin pregnancies to allow a comparison to the current national population.

3.3 Discussion

The study population was composed of 639 stillbirths, 179 late miscarriages and 246 early miscarriages, totalling 1,064 autopsies for evaluation. The majority of deaths (766, 72%) were antepartum of which 82% had an intrauterine retention time of at least 24 hours. There were more male fetuses in the study population, but this was not statistically significant.

The maternal demographics of the study population were explored in great detail. Firstly, it was found that the study population (in total and separately for both miscarriages and stillbirths) was significantly older than the distribution of all pregnancies nationally, in keeping with published literature; an interesting find as 2011 saw the largest percentage increase in fertility in women 40 years and older since 2001 in England and Wales (52, 97, 191). The finding confirms the association of increasing maternal age with increased risk of intrauterine demise.

Secondly, the majority of mothers in the study population were white but black mothers were significantly overrepresented in the study population compared to data from national statistics, in keeping with published data that stillbirth risk is increased in mothers of African or Asian ethnicity (94, 95, 186, 192). Furthermore, among women who suffer an intrauterine death, white mothers appeared more predisposed to stillbirth and black mothers to miscarriage the possible causes of which are explored in other chapters.

The mothers in the study population were also significantly more overweight and obese than the unselected national population (indicating that overweight is associated with intrauterine death risk) but interestingly there was no significant difference in BMI between mothers of miscarriages and stillbirths. This suggests that

a raised maternal BMI is associated similarly with both fetal loss and stillbirth across all gestations (49, 52).

The majority of mothers in the study population were primigravida (another known association to stillbirth) and the primigravida mothers in the present study were more likely to have a stillbirth than miscarriage (49, 52). However those mothers with an obstetric history of vaginal bleeding during pregnancy, uterine fibroids, in vitro fertilization or history of a previous fetal loss, termination or ectopic pregnancy were more likely affected by miscarriage.

Within the present study, most mothers were non-smokers, did not drink alcohol or take illicit drugs during their pregnancy and did not suffer from infection. Of the small number of mothers who suffered from some form of diabetes or hypertension during pregnancy, these mothers had significantly more stillbirths than miscarriages. Mothers with pre-eclampsia also had significantly more stillbirths than miscarriages in comparison to mothers with either chronic hypertension or pregnancy induced hypertension.

In conclusion, this chapter confirms the known associations to stillbirth and miscarriage of rising maternal age, increased maternal BMI, maternal ethnicity, diabetes and hypertension but also provides new data suggesting;

1. White mothers appear more predisposed to stillbirth and black mothers to miscarriage
2. A raised maternal BMI is associated with fetal loss but not significantly more associated with a miscarriage or a stillbirth loss
3. Mothers who are primigravida appear more predisposed to stillbirth than miscarriage

4. Mother with an obstetric history of vaginal bleeding during pregnancy, uterine fibroids and in vitro fertilization are more predisposed to miscarriages than stillbirth

Further research is recommended to analyse the scientific and clinical reasons for such demographic associations with miscarriage and stillbirth and measures that could be taken to address such factors.

4. Cause of death

4.0 Background

4.1 Chapter Aims

4.2 Methods

4.3 Classifying causes of death

4.4 Cause of death

- 4.4.1 All causes of death
- 4.4.2 Causes of death in cases of Early Miscarriage
- 4.4.3 Causes of death in Cases of Late miscarriage
- 4.4.4 Causes of death in cases of Stillbirth

4.5 Maternal Ethnicity and cause of death

- 4.5.1 Maternal ethnicity and cause of death in all cases
- 4.5.2 Maternal ethnicity and cause of death in early miscarriage
- 4.5.3 Maternal ethnicity and cause of death in late miscarriage
- 4.5.4 Maternal ethnicity and cause of death in stillbirth

4.6 Maternal Age and cause of death

- 4.6.1 Maternal age and cause of death in all cases
- 4.6.2 Maternal age and cause of death in cases of early miscarriage
- 4.6.3 Maternal age and cause of death bin cases of late miscarriage
- 4.6.4 Maternal age and cause of death in cases of stillbirth

4.7 Maternal Body Mass Index (BMI) and cause of death

- 4.7.1 Maternal BMI and cause of death in all cases
- 4.7.2 Maternal BMI and cause of death in cases of early miscarriage
- 4.7.3 Maternal BMI and cause of death bin cases of late miscarriage
- 4.7.4 Maternal age and cause of death in cases of stillbirth

4.8 Maternal Diabetes Mellitus and cause of death

4.9 Maternal Hypertension and cause of death

4.10 Fetal Maceration and cause of death

4.11 Postmortem interval and cause of death

4.12 ReCoDe classification

4.13 How often can the autopsy examination identify a specific cause of death?

4.14 Discussion

4.0 Background

The underlying aim of autopsy investigation of stillbirth is to determine the cause and mechanism of death, for parental closure, management of subsequent pregnancies and development of potential future interventions. Over the last 60 years there have been many attempts to classify the causes of death in stillbirths but according to which classification system is used, (of which there are over 30 (193)), 15-60% of all stillbirths remain unexplained, despite postmortem examinations being undertaken by trained professionals in specialist centres (4, 5). Four of the most well-known classification systems include;

1. The Aberdeen system, predominantly based on obstetric findings and clinical history rather than the findings at autopsy (111-113)
2. The Wigglesworth system which subdivides cases into general groups with clear implications for clinical management (4)
3. Relevant Condition at Death (ReCoDe) system which aims to identify underlying risk factors, not necessarily mechanisms and incorporates growth restriction as a cause of death (5)
4. Causes of death and associated conditions system (CODAC), which is designed to accommodate both the main cause of death as well as associated conditions and combines both the clinical and pathological causes of stillbirth (114).

A review article published in 2009 evaluated the use of the above classification systems, as well as others such as the PSANZ-PDC (Perinatal Society of Australia and New Zealand – Perinatal Death Classification) and TULIP, and recommended that The Extended Wigglesworth and the Amended Aberdeen classification systems should no longer be used. The best performing system in terms of ease of use, inter-

observer agreement and achieving the lowest rate of "unexplained" causes was CODAC followed by PSANZ-PDC and ReCoDe (115). However, all of these systems are highly subjective and allow for observer bias, making accurate comparisons between systems challenging. Furthermore, whilst it is tempting to suggest that reducing "unexplained" cases is beneficial, this is only the case if there is clear evidence that the "cause" assigned is correct; in most systems this is not the case as many "causes" of death are based on associations and risk factors and may therefore be spurious.

4.1 Chapter aims

The aim of this chapter is therefore to use a large dataset, from two specialist centres, to eliminate observer bias as far as possible, by objectively assigning causes and classifications of death in each case based on predetermined criteria, to facilitate the analysis and review of:

1. The causes of death within stillbirth and intrauterine death populations,
2. The associations of maternal demographic features with cause of fetal death including,
    a. Maternal ethnicity
    b. Maternal age
    c. Maternal BMI
    d. Maternal Diabetes
    e. Maternal Hypertension.
3. The effects of maceration and postmortem interval on assigned cause of death

4. A comparison of the distribution of causes of death with a well-known classification system, ReCoDe.
5. Evidence to determine the frequency with which autopsy reliably provides a definite cause of death in stillbirth.

4.2 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriage from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Data was analysed through queries and statistical tests run using Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Statistical tests can be viewed in detail in Appendix 3.

4.3 Classifying causes of death

For the purposes of this study, predetermined criteria were used to assign an objective cause of death to each case based on the antenatal details provided and autopsy findings. As far as possible, definitions of causes of death were based on objective and documented findings, listed below. (See Appendix 4 for details of the 'other' causes of death assigned by the original pathologist that were then reassigned to specific, objective causes of death).

- *Abruption*: Definite clinical history of abruption +/- concurrent placental or autopsy findings

- *Ascending Infection:* Histologically proven chorioamnionitis +/- funisitis+/- fetal pneumonia (it is accepted that in such cases it may be impossible to determine whether ascending infection was primary or secondary).

- *Birth Trauma:* A documented complication that occurred during delivery leading to fatal intrapartum events, with consistent, if non-diagnostic autopsy findings e.g. shoulder dystocia.

- *Congenital abnormalities:* Congenital abnormalities documented antenatally or at autopsy, which likely accounted for the death.
- *Feto-Maternal Haemorrhage (FMH)*: Documented abnormal Kleihauer test, with our without autopsy features consistent with severe FMH.

- *Infection:* Documented systemic maternal infection associated with fetal death.

- *Known cord accident:* Witnessed and recorded cord complication during delivery – e.g. cord prolapse (the simple finding of a cord knot or abnormal coiling at autopsy in the absence of specific clinical history or other findings were not included in this category; see below).

- *Known IUGR:* IUGR detected antenatally and with no other specific cause of death found at autopsy

- *Placenta:* Significant placental pathology confirmed with definite or unequivocal abnormal histological features which likely caused the death e.g. severe uteroplacental malperfusion, chronic histiocytic intervillositis (placental histological changes of uncertain significance were not recorded here; see below).

- *Pre-term:* Likely cervical incompetence/ idiopathic severe preterm labour resulting in intrapartum death from antenatal history

- *Twin complication:* Documented complication of twinning e.g. twin to twin transfusion syndrome

- *Unexplained lesion, baby*:* Unexplained cause of death but with the presence of a fetal finding of unknown significance e.g. mild intraventricular haemorrhage

- *Unexplained lesion, clinical*:* Unexplained cause of death, but with the presence of a clinical risk factor of unknown significance in the specific case e.g. maternal cholestasis

- *Unexplained lesion, cord*:* Unexplained cause of death, but with the presence of a cord finding of unknown significance e.g. true cord knot but with no thrombosis or other pathological findings.

- *Unexplained lesion, Placenta**: Unexplained cause of death, but with the presence of placental findings of unknown significance e.g. increased syncytial knots but without changes of severe malperfusion, intervillous thrombus etc.

- *Unexplained obese:* Unexplained cause of death, but with the presence of documented maternal obesity (BMI>30).

- *Unexplained post-term*: Unexplained cause of death, but the presence of documented post-term delivery (post 41 weeks).

- *Unexplained with previous fetal loss*: Unexplained cause of death, but with the presence of a maternal history of previous fetal loss.

- *Unexplained with Diabetes:* Unexplained cause of death, but with the presence of some form of diabetes Mellitus (either Diabetes Mellitus or Gestational Diabetes)..

- *Unexplained, IUGR:* Unexplained cause of death, with no history of antenatal IUGR but the presence of definite IUGR diagnosed at autopsy. This category does not include those Small for Gestational Age cases found during analysis based purely on biometry with no associated histological abnormalities - these results are to be discussed separately in Chapter 5.

- *Unexplained, unexplained:* No cause of death found at autopsy, no abnormal placental findings and no clinical risk factor associations.

- *Unexplained all:* No cause of death found based on antenatal history and autopsy findings. This category encompasses all of the "unexplained" causes of death listed above.

* In the simplified classification system these groups were placed together into the unexplained lesion category.

The effects of growth restriction and fetuses that are small for gestational age (SGA) are discussed in detail in Chapter 5. SGA is only considered in this chapter when comparing causes of death in the current study population using this classification with the ReCoDe classification system.

4.4 Causes of death

4.4.1 Cause of Death: overall, all cases (miscarriage and stillbirth)

Cause of death- all cases	Number / percentage of cases
Abruption	**38 (4%)**
Ascending Infection	**176 (17%)**
Birth trauma	**7 (1%)**
Congenital abnormalities	**50 (5%)**
Feto-maternal Haemorrhage	**6 (1%)**
Infection	**13 (1%)**
Known cord accident	**4 (<1%)**
Known IUGR	**18 (2%)**
Placenta	**56 (5%)**
Pre-eclampsia	**16 (2%)**
Preterm	**4 (<1%)**
Twin Complication	**21 (2%)**
Unexplained (all)	**655 (62%)**
• Unexplained lesion, baby	20 (2%)
• Unexplained lesion, clinical	16 (2%)
• Unexplained lesion, cord	13 (1%)
• Unexplained lesion, Placenta	78 (7%)
• Unexplained obese	45 (4%)
• Unexplained obese and post-term	5 (<1%)
• Unexplained Obese and previous fetal loss	26 (2%)
• Unexplained obese and GDM	5 (<1%)
• Unexplained obese and IDDM	2 (<1%)
• Unexplained post-term	29 (3%)
• Unexplained, previous fetal loss	100 (19%)
• Unexplained, unexplained	292 (27%)
• Unexplained with GDM	14 (1%)
• Unexplained with IDDM	7 (1%)
• Unexplained with GDM and previous fetal loss	1 (<1%)
• Unexplained with IDDM and previous fetal loss	1 (<1%)
• Unexplained with IUGR at PM	1 (<1%)
Total:	**1064**

Table 42 All causes of death in the total study population

The most common category was unexplained (63%), with 44% of these cases being unexplained, unexplained. The second most common cause of death overall was ascending infection (17%), but this includes midtrimester miscarriages.

Simplified Cause of death- Overall	Number / percentage of cases
Abruption	**38 (4%)**
Ascending Infection	**176 (17%)**
Birth trauma	**7 (1%)**
Congenital abnormalities	**50 (5%)**
Feto-maternal Haemorrhage	**6 (1%)**
Infection	**13 (1%)**
Known cord accident	**4 (<1%)**
Known IUGR	**18 (2%)**
Placenta	**56 (5%)**
Pre-eclampsia	**16 (2%)**
Preterm	**4 (<1%)**
Twin Complication	**21 (2%)**
Unexplained (all)	**655 (62%)**
• Unexplained lesion	127 (12%)
• Unexplained obese	83 (8%)
• Unexplained post-term	29 (3%)
• Unexplained, previous fetal loss	100 (9%)
• Unexplained, unexplained	292 (27%)
• Unexplained with Diabetes	24 (2%)
• Unexplained with IUGR at PM	1 (<1%)
Total:	**1064**

Table 43 Simplified overall causes of death

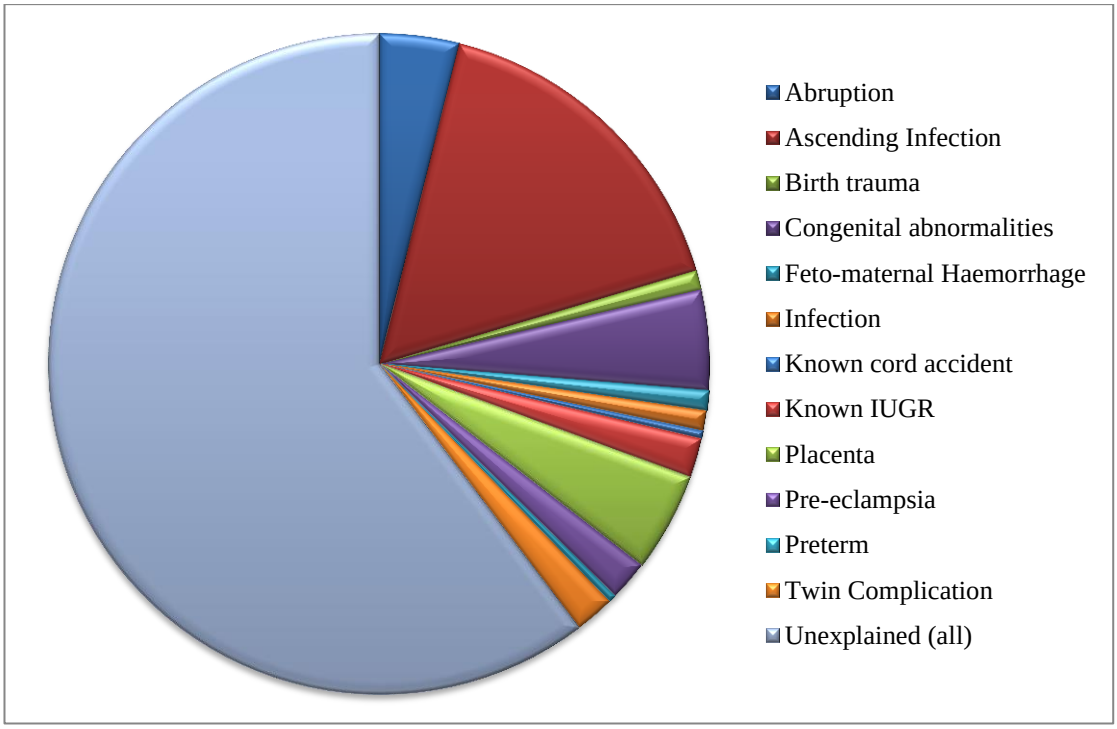

Figure 23 *All causes of death*

4.4.2 Cause of death Early Miscarriage

Cause of death – Early miscarriage	Number of cases
Abruption	**3 (1%)**
Ascending Infection	**58 (24%)**
Birth trauma	**0 (0%)**
Congenital abnormalities	**9 (4%)**
Feto-maternal Haemorrhage	**0 (0%)**
Infection	**0 (0%)**
Known cord accident	**1 (<1%)**
Known IUGR	**4 (2%)**
Placenta	**1 (<1%)**
Pre-eclampsia	**0 (0%)**
Preterm	**0 (0%)**
Twin Complication	**8 (3%)**
Unexplained (all)	**162 (66%)**
• Unexplained lesion, baby	1 (<1%)
• Unexplained lesion, clinical	1 (<1%)
• Unexplained lesion, cord	1 (<1%)
• Unexplained lesion, Placenta	16 (7%)
• Unexplained obese	13 (5%)
• Unexplained obese and post-term	0 (0%)
• Unexplained Obese and previous fetal loss	14 (6%)
• Unexplained obese and GDM	0 (0%)
• Unexplained obese and IDDM	0 (0%)
• Unexplained post-term	0 (0%)
• Unexplained, previous fetal loss	43 (17%)
• Unexplained unexplained	69 (28%)
• Unexplained with GDM	1 (<1%)
• Unexplained with IDDM	2 (1%)
• Unexplained with GDM and previous fetal loss	1 (<1%)
• Unexplained with IDDM and previous fetal loss	0 (0%)
• Unexplained with IUGR	0 (0%)
Total:	246

Table 44 Cause of death in cases of early miscarriage

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Simplified Cause of death – Early miscarriage	Number of cases
Abruption	**3 (1%)**
Ascending Infection	**58 (24%)**
Birth trauma	**0 (0%)**
Congenital abnormalities	**9 (4%)**
Feto-maternal Haemorrhage	**0 (0%)**
Infection	**0 (0%)**
Known cord accident	**1 (<1%)**
Known IUGR	**4 (2%)**
Placenta	**1 (<1%)**
Pre-eclampsia	**0 (0%)**
Preterm	**0 (0%)**
Twin Complication	**8 (3%)**
Unexplained (all)	**162 (66%)**
• Unexplained lesion	19 (8%)
• Unexplained obese	27 (11%)
• Unexplained post-term	0 (0%)
• Unexplained, previous fetal loss	43 (17%)
• Unexplained unexplained	70 (28%)
• Unexplained with Diabetes	4 (2%)
• Unexplained with IUGR	0 (0%)
Total:	**246**

Table 45 Simplified cause of death early miscarriage

4.4.3 Cause of death Late Miscarriage

Cause of death – Late miscarriage	Number of cases
Abruption	**5 (3%)**
Ascending Infection	**59 (33%)**
Birth trauma	**0 (0%)**
Congenital abnormalities	**5 (3%)**
Feto-maternal Haemorrhage	**0 (0%)**
Infection	**1 (1%)**
Known cord accident	**1 (1%)**
Known IUGR	**5 (3%)**
Placenta	**4 (2%)**
Pre-eclampsia	**0 (0%)**
Preterm	**3 (2%)**
Twin Complication	**4 (2%)**
Unexplained (all)	**92 (51%)**
• Unexplained lesion, baby	5 (3%)
• Unexplained lesion, clinical	0 (0%)
• Unexplained lesion, cord	0 (0%)
• Unexplained lesion, Placenta	5 (3%)
• Unexplained obese	6 (3%)
• Unexplained obese and post-term	0 (0%)
• Unexplained Obese and previous fetal loss	2 (1%)
• Unexplained obese and GDM	0 (0%)
• Unexplained obese and IDDM	1 (1%)
• Unexplained post-term	0 (0%)
• Unexplained, previous fetal loss	16 (9%)
• Unexplained unexplained	54 (30%)
• Unexplained with GDM	1 (1%)
• Unexplained with IDDM	2 (1%)
• Unexplained with GDM and previous fetal loss	0 (0%)
• Unexplained with IDDM and previous fetal loss	0 (0%)
• Unexplained with IUGR	0 (0%)
Total:	**179**

Table 46 Causes of death in cases of late miscarriage

Simplified Cause of death – Late miscarriage	Number of cases
Abruption	**5 (3%)**
Ascending Infection	**59 (33%)**
Birth trauma	**0 (0%)**
Congenital abnormalities	**5 (3%)**
Feto-maternal Haemorrhage	**0 (0%)**
Infection	**1 (1%)**
Known cord accident	**1 (1%)**
Known IUGR	**5 (3%)**
Placenta	**4 (2%)**
Pre-eclampsia	**0 (0%)**
Preterm	**3 (2%)**
Twin Complication	**4 (2%)**
Unexplained (all)	**92 (51%)**
• Unexplained lesion	10 (6%)
• Unexplained obese	9 (5%)
• Unexplained post-term	0 (0%)
• Unexplained, previous SB	16 (9%)
• Unexplained unexplained	54 (31%)
• Unexplained with Diabetes	3 (1%)
• Unexplained with IUGR	0 (0%)
Total:	**179**

Table 47 Simplified cause of death, late miscarriage

4.4.4 Cause of death Stillbirth

Cause of death - Stillbirth	Number of cases
Abruption	**30 (5%)**
Ascending Infection	**59 (9%)**
Birth trauma	**7 (1%)**
Congenital abnormalities	**36 (6%)**
Feto-maternal Haemorrhage	**6 (1%)**
Infection	**12 (2%)**
Known cord accident	**2 (<1%)**
Known IUGR	**9 (1%)**
Placenta	**51 (8%)**
Pre-eclampsia	**16 (3%)**
Preterm	**1 (<1%)**
Twin Complication	**9 (<1%)**
Unexplained (all)	**401 (63%)**
• Unexplained lesion, baby	14 (2%)
• Unexplained lesion, clinical	15 (2%)
• Unexplained lesion, cord	12 (2%)
• Unexplained lesion, Placenta	57 (9%)
• Unexplained obese	26 (4%)
• Unexplained obese and post-term	5 (1%)
• Unexplained Obese and previous fetal loss	10 (2%)
• Unexplained obese and GDM	5 (1%)
• Unexplained obese and IDDM	1 (<1%)
• Unexplained post-term	29 (5%)
• Unexplained, previous fetal loss	41 (6%)
• Unexplained unexplained	169 (26%)
• Unexplained with GDM	12 (2%)
• Unexplained with IDDM	3 (<1%)
• Unexplained with GDM and previous fetal loss	0 (0%)
• Unexplained with IDDM and previous fetal loss	1 (<1%)
• Unexplained with IUGR	1 (<1%)
Total:	**639**

Table 48 Causes of death for cases of stillbirth

Simplified Cause of death - Stillbirth	Number of cases
Abruption	**30 (5%)**
Ascending Infection	**59 (9%)**
Birth trauma	**7 (1%)**
Congenital abnormalities	**36 (6%)**
Feto-maternal Haemorrhage	**6 (1%)**
Infection	**12 (2%)**
Known cord accident	**2 (<1%)**
Known IUGR	**9 (1%)**
Placenta	**51 (8%)**
Pre-eclampsia	**16 (3%)**
Preterm	**1 (<1%)**
Twin Complication	**9 (1%)**
Unexplained (all)	**401 (63%)**
• Unexplained lesion	98 (15%)
• Unexplained obese	47 (7%)
• Unexplained post-term	29 (5%)
• Unexplained, previous SB	41 (6%)
• Unexplained unexplained	169 (26%)
• Unexplained with Diabetes	16 (3%)
• Unexplained with IUGR	1 (<1%)
Total:	**639**

Table 49 Simplified Cause of death, stillbirth

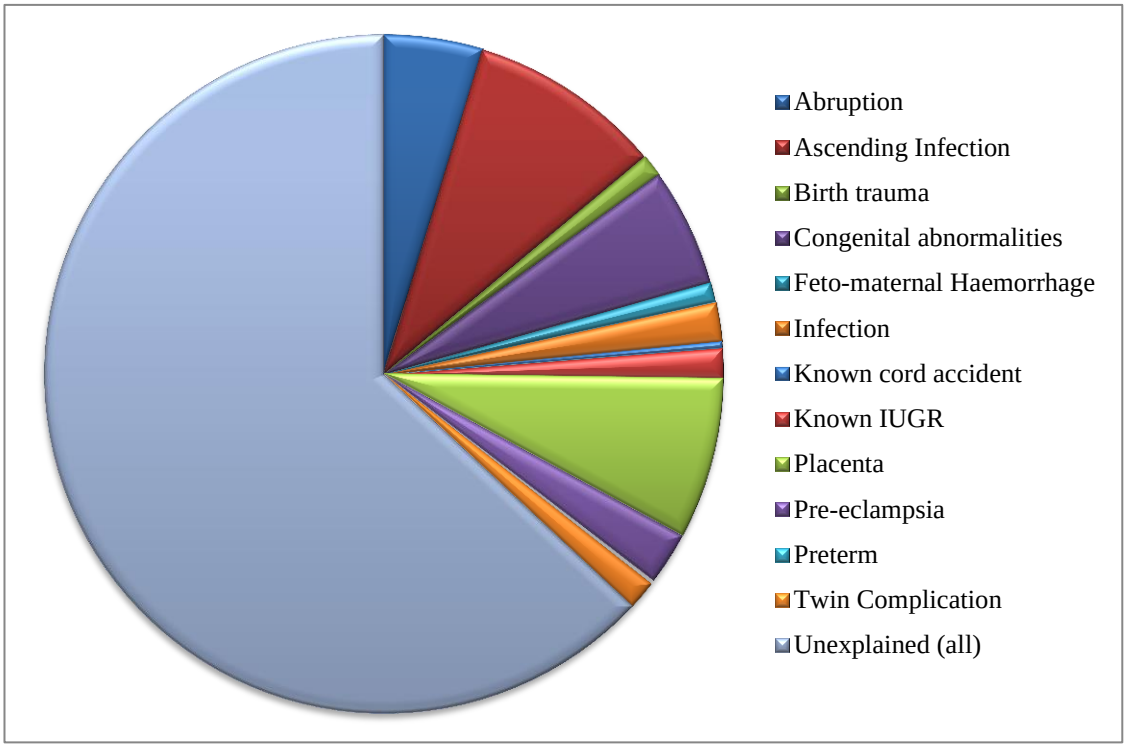

Figure 24 *Simplified Cause of death, stillbirth*

4.4.5 Comparison of miscarriage and stillbirth causes of death

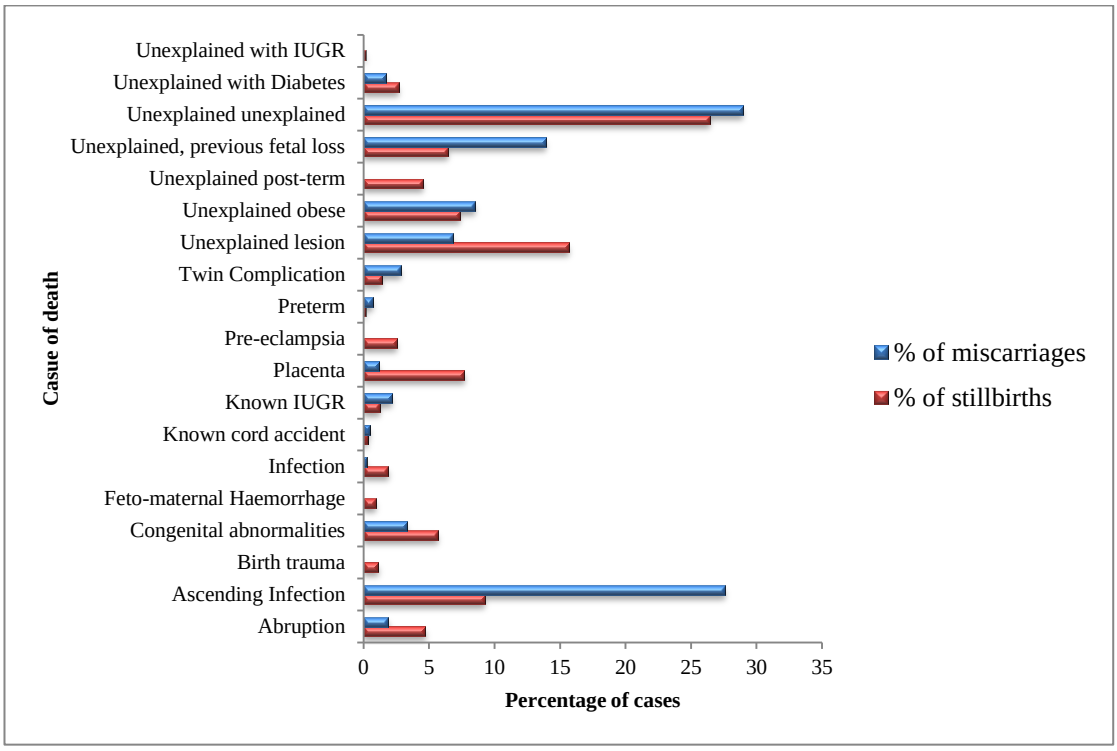

Figure 25 *Simplified causes of death in cases of miscarriage and stillbirth*

Unexplained, unexplained cases represented the largest category in both cohorts. Placental abruption, maternal infection, placental abnormalities and unexplained cases with lesions were statistically more common in stillbirths (z=2.421, p= 0.0155; z= 2.389, p =0.0169; z=4.725, p<0.0001; z=4.32, p<0.0001 respectively).

Ascending Infection and unexplained death with a previous history of fetal loss were statistically more common in miscarriages (z=7.867, p <0.0001 and z= 4.088, p<0.0001 respectively).

There were no other significant differences between the remaining causes of death.

There were only 24 cases (19 antepartum and five intrapartum deaths) in which there were no significant clinical findings such as overweight, obese or underweight BMI; cholestasis; maternal hypertension; diabetes mellitus; previous history of fetal loss or

termination of pregnancy; abnormal cervical smears or treatment to the cervix and no other significant maternal past medical history. Of these 24 cases there were:

- Nine miscarriages
- 15 stillbirths

The causes of death in the 15 cases of stillbirth are detailed below (*Table* 50).

Cause of death	Number of cases
Ascending infection	4 (17%)
Placenta	1 (4%)
Unexplained	19 (79%)
Total:	**24**

Table 50 Causes of death in cases with no significant antenatal history

These findings suggest that if there is no significant clinical history, an autopsy is unlikely to yield a positive finding in nearly 80% of cases and in the remaining 20% the cause of death will be found on examination of the placenta.

4.4.6 Limited versus complete autopsy

81 cases within the study had a limited autopsy. *Table* 51 details the degree of limitation and shows that in most cases the majority still had an unexplained death. In total, 63% of all limited postmortems had an unexplained death; the same as the overall percentage of unexplained death (62%) and thus for the analysis below all autopsies (both limited and complete) have been examined as one group.

	Number of cases	Number of unexplained causes of death	% unexplained of those with limited PM
Limited to Abdomen, chest and head	1	1	100
No Brain examination	9	4	44
Limited to external+/- MRI+/- Placenta	52	37	71
Limited to chest and abdomen	4	1	25
Limited other	7	4	57
Laproscopic autopsy+/- MRI	7	3	43
No histology to be taken	1	1	100
Total:	**81**	**51**	**63**

Table 51 Unexplained causes of death in limited autopsies

4.5 Maternal Ethnicity and Cause of Death

4.5.1 Maternal ethnicity and cause of death in all cases

Cause of death - all	White	Mixed/ oriental	Asian	Black	**Total:**
Abruption	15 (3%)	0 (0%)	5 (9%)	7 (3%)	**27 (4%)**
Ascending Infection	48 (10%)	3 (17%)	12 (21%)	69 (33%)	**132 (18%)**
Birth trauma	2 (<1%)	0 (0%)	1 (2%)	0 (0%)	**3 (<1%)**
Congenital abnormalities	18 (4%)	0 (0%)	2 (3%)	7 (3%)	**27 (4%)**
Feto-maternal Haemorrhage	3 (1%)	1 (6%)	0 (0%)	0 (0%)	**4 (1%)**
Infection	6 (1%)	2 (11%)	0 (0%)	1 (<1%)	**9 (1%)**
Known cord accident	3 (1%)	0 (0%)	0 (0%)	1 (<1%)	**4 (1%)**
Known IUGR	10 (2%)	0 (0%)	3 (5%)	2 (1%)	**15 (2%)**
Placenta	35(7%)	0 (0%)	3 (5%)	7 (7%)	**45 (6%)**
Pre-eclampsia	5 (1%)	0 (0%)	0 (0%)	5 (2%)	**10 (1%)**
Preterm	0 (0%)	0 (0%)	0 (0%)	3 (1%)	**3 (<1%)**
Twin Complication	9 (2%)	0 (0%)	0 (0%)	2 (1%)	**11 (1%)**
Unexplained (all)	315 (67%)	12 (67%)	32 (55%)	103 (50%)	**462(61%)**
Total	**469 (62%)**	**18 (2%)**	**58 (8%)**	**207 (28%)**	**752**

Table 52 Cause of death, (all cases) by maternal ethnicity

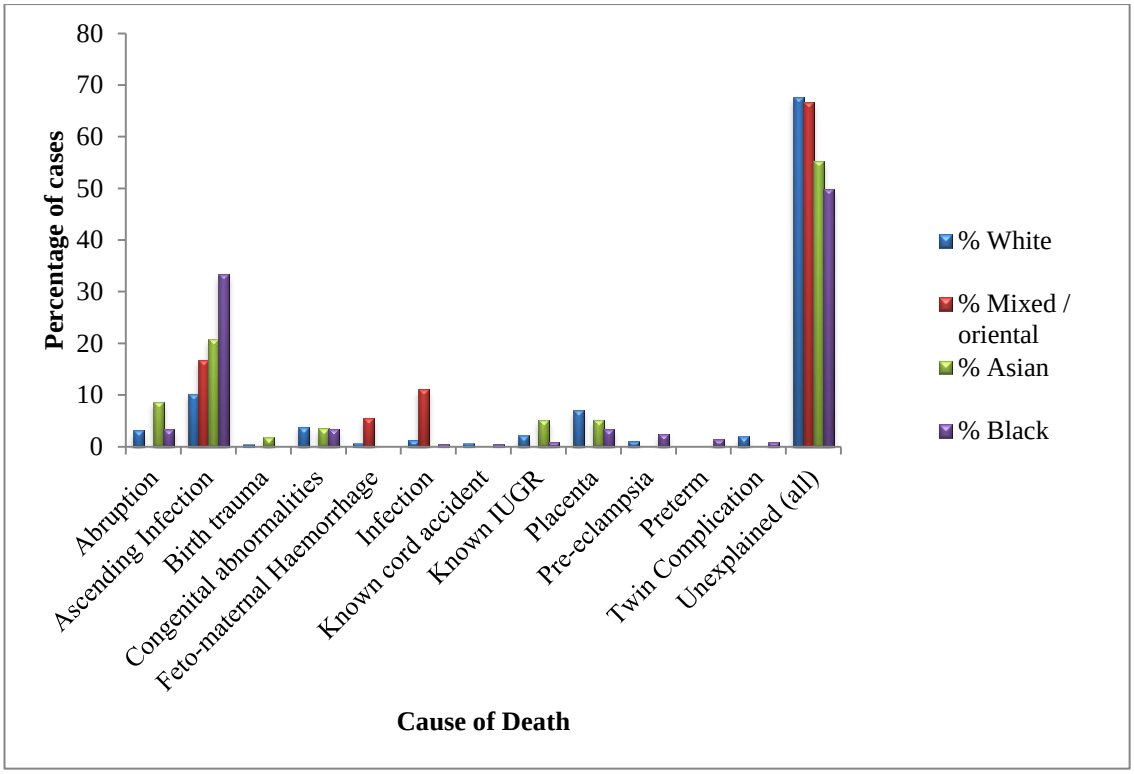

Figure 26 *Cause of death by maternal ethnicity*

The majority of cases were unexplained for all ethnic groups, followed by ascending infection.

Black and Asian mothers had significantly greater proportion of ascending infection than white mothers ($z=7.352$, $p<0.0001$ and $z=2.365$, $p = 0.08$ respectively)

White mothers had significantly greater proportion of unexplained deaths than all other ethnicities ($z=4.101$, $p<0.0001$) but the same proportion of unexplained deaths as mothers of oriental/mixed ethnicity.

4.5.2 Maternal ethnicity and cause of death in cases of early miscarriage

Cause of death – Early Miscarriage	White	Mixed/ oriental	Asian	Black	Total:
Abruption	2 (2%)	0 (0%)	0 (0%)	0 (0%)	**2 (1%)**
Ascending Infection	10 (11%)	1 (20%)	5 (36%)	24 (44%)	**40 (25%)**
Birth trauma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	2 (2%)	0 (0%)	0 (0%)	1 (2%)	**3 (2%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known cord accident	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1 (1%)**
Known IUGR	1 (1%)	0 (0%)	1 (7%)	1 (2%)	**3 (2%)**
Placenta	0 (0%)	0 (0%)	0 (0%)	1 (2%)	**1 (1%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Preterm	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Twin Complication	2 (2%)	0 (0%)	0 (0%)	2 (4%)	**4 (2%)**
Unexplained (all)	69 (79%)	4 (80%)	8 (57%)	26 (47%)	**107 (66%)**
Total	**87**	**5**	**14**	**55**	**161**

Table 53 Cause of death in early miscarriage by maternal Ethnicity – excludes 85 unknown ethnicities

4.5.3 Maternal ethnicity and cause of death in cases of late miscarriage

Cause of death – late miscarriage	White	Mixed/ oriental	Asian	Black	**Total**
Abruption	2 (3%)	0 (0%)	1 (10%)	1 (2%)	**4 (3%)**
Ascending Infection	18 (25%)	2 (100%)	4 (40%)	25 (48%)	**49 (36%)**
Birth trauma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	3 (4%)	0 (0%)	0 (0%)	0 (0%)	**3(2%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known cord accident	0 (0%)	0 (0%)	0 (0%)	1 (2%)	**1 (1%)**
Known IUGR	4 (6%)	0 (0%)	1 (10%)	0 (0%)	**5 (4%)**
Placenta	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1(1%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Preterm	0 (0%)	0 (0%)	0 (0%)	2 (4%)	**2 (1%)**
Twin Complication	2 (3%)	0 (0%)	0 (0%)	0 (0%)	**2 (1%)**
Unexplained (all)	41 (58%)	0 (0%)	4 (40%)	23 (44%)	**68 (50%)**
Total	**71**	**2**	**10**	**52**	**135**

Table 54 Cause of death in late miscarriage by maternal Ethnicity – excludes 45 unknown ethnicities

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4.5.4 Maternal ethnicity and cause of death in cases of stillbirth

Cause of death –Stillbirth	White	Mixed/ Oriental	Asian	Black	**Total**
Abruption	11 (4%)	0 (0%)	4 (12%)	6 (6%)	**21 (5%)**
Ascending Infection	20 (6%)	0 (0%)	3 (9%)	20 (20%)	**43 (9%)**
Birth trauma	2 (1%)	0 (0%)	1 (3%)	0 (0%)	**3 (1%)**
Congenital abnormalities	13 (4%)	0 (0%)	2 (6%)	6 (6%)	**21 (5%)**
Feto-maternal Haemorrhage	3 (1%)	1 (9%)	0 (0%)	0 (0%)	**4 (1%)**
Infection	6 (2%)	2 (18%)	0 (0%)	1 (1%)	**9 (2%)**
Known cord accident	2 (1%)	0 (0%)	0 (0%)	0 (0%)	**2 (<1%)**
Known IUGR	5 (2%)	0 (0%)	1 (3%)	0 (0%)	**6 (1%)**
Placenta	34 (11%)	0 (0%)	3 (9%)	6 (6%)	**43 (9%)**
Pre-eclampsia	5 (2%)	0 (0%)	0 (0%)	5 (5%)	**10 (2%)**
Preterm	0 (0%)	0 (0%)	0 (0%)	1 (1%)	**1 (<1%)**
Twin Complication	5 (2%)	0 (0%)	0 (0%)	0 (0%)	**5 (1%)**
Unexplained (all)	205 (66%)	8 (73%)	20 (59%)	54 (55%)	**287 (63%)**
Total:	**311**	**11**	**34**	**99**	**455**

Table 55 Cause of death in stillbirth by maternal ethnicity – excludes 184 unknown ethnicities

4.6 Maternal Age and cause of death

4.6.1 Maternal age and cause of death all cases

Cause of death	Maternal age less than and equal to 35 years	Maternal age 36 -40 years	Maternal age more than or equal to 41 years	**Total**
Abruption	28 (4%)	7 (4%)	1 (2%)	**36 (3%)**
Ascending infection	130 (16%)	36 (19%)	8 (17%)	**174 (17%)**
Birth Trauma	6 (1%)	0 (0%)	0 (0%)	**6 (1%)**
Congenital abnormalities	39 (5%)	6 (3%)	4 (8%)	**49 (5%)**
Feto-maternal Hameorrhage	4 (1%)	1 (1%)	1 (2%)	**6 (1%)**
Infection	11 (1%)	1 (1%)	1 (2%)	**13 (1%)**
Known cord accident	4 (1%)	0 (0%)	0 (0%)	**4 (<1%)**
Known IUGR	14 (2%)	3 (2%)	1 (2%)	**18 (2%)**
Placenta	42 (5%)	8 (4%)	6 (13%)	**56 (5%)**
Pre-eclampsia	14 (2%)	2 (1%)	0 (0%)	**16 (2%)**
Pre-term	4 (1%)	0 (0%)	0 (0%)	**4 (<1%)**
Twin complications	16 (2%)	4 (2%)	1 (2%)	**21 (2%)**
Unexplained (all)	486 (61%)	125 (65%)	25 (52%)	**636 (61%)**
Total:	**798 (77%)**	**193 (19%)**	**48 (5%)**	**1039**

Table 56 Cause of death by maternal age (Excludes 26 cases with no maternal age)

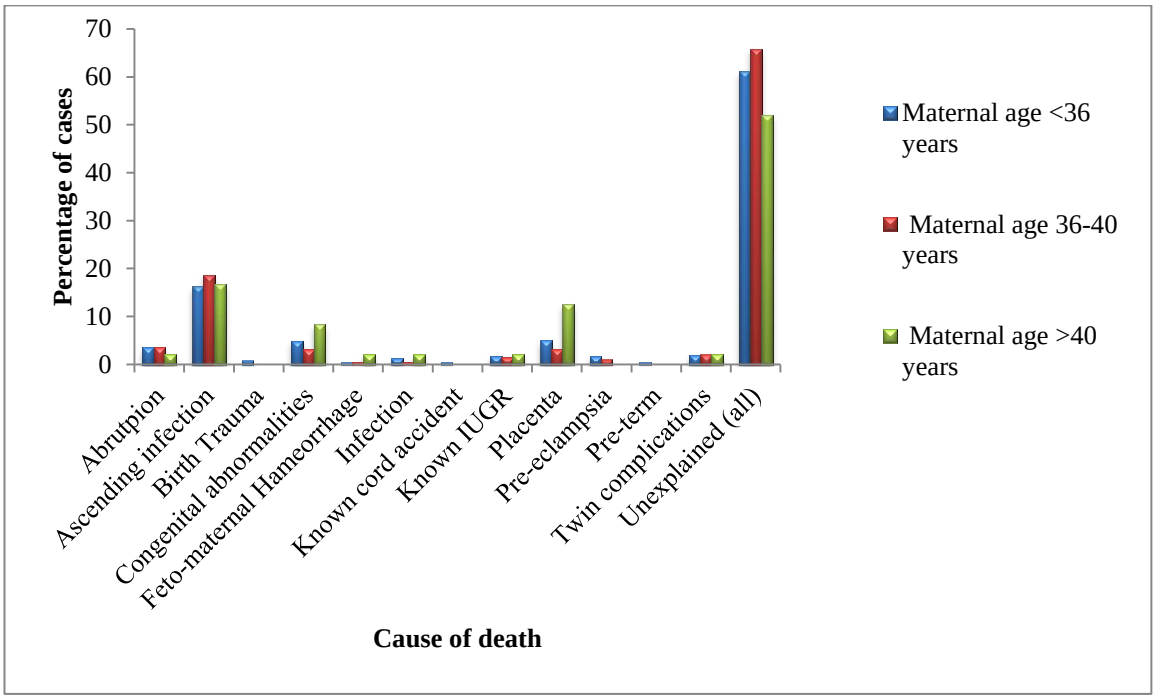


Figure 27 *Maternal age and cause of death*

The majority of the study population (77%) had a maternal age less than or equal to age 35 years. The most common cause of death remained unexplained (not significantly different between age groups z= 1.329, p =0.1835), followed by ascending infection. Mothers over the age of 40 had a significantly greater proportion of placental causes of death than mothers <40 years of age (z=2.234, p=0.0255).

4.6.2 Maternal age and cause of death in cases of Early Miscarriage

Cause of death	Maternal age less than or equal to 35 years	Maternal age 36 -40 years	Maternal age more than or equal to 41 years	**Total**
Abruption	3 (2%)	0 (0%)	0 (0%)	**3 (1%)**
Ascending infection	45 (26%)	13 (22%)	0 (0%)	**58 (24%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	6 (4%)	3 (5%)	0 (0%)	**9 (4%)**
Feto-maternal Hameorrhage	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known cord accident	1 (1%)	0 (0%)	0 (0%)	**1 (<1%)**
Known IUGR	3(2%)	0 (0%)	1 (10%)	**4 (2%)**
Placenta	1 (1%)	0 (0%)	0 (0%)	**1 (<1%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Pre-term	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Twin complications	6 (0%)	2 (3%)	0 (0%)	**8 (3%)**
Unexplained (all)	105 (62%)	42 (70%)	9(90%)	**156 (65%)**
Total:	**170 (71%)**	**60 (25%)**	**10 (4%)**	**240**

Table 57 Cause and death and maternal age, early miscarriage (excludes 6 cases with no maternal age)

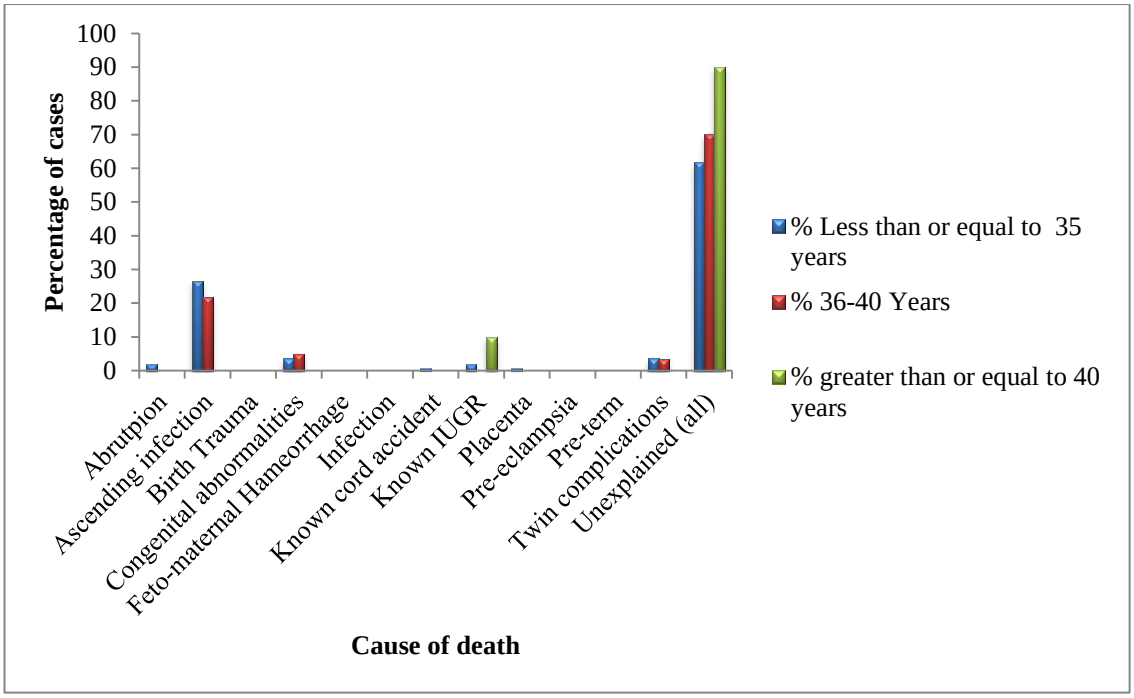


Figure 28 *Cause of death in early miscarriage by maternal age*

4.6.3 Maternal age and cause of death in cases of Late miscarriage

Cause of death	Maternal age less than and+ equal to 35 years	Maternal age 36 - 40 years	Maternal Age more than or equal to 41years	**Total**
Abrutpion	5 (4%)	0 (0%)	0 (0%)	**5 (3%)**
Ascending infection	39 (29%)	12 (39%)	7 (54%)	**58 (3%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	4 (3%)	0 (0%)	1 (8%)	**5 (3%)**
Feto-maternal Hameorrhage	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	1 (1%)	0 (0%)	0 (0%)	**1 (1%)**
Known cord accident	1 (1%)	0 (0%)	0 (0%)	**1 (1%)**
Known IUGR	5 (4%)	0 (0%)	0 (0%)	**5 (3%)**
Placenta	4 (3%)	0 (0%)	0 (0%)	**4 (2%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Pre-term	3 (2%)	0 (0%)	0 (0%)	**3 (2%)**
Twin complications	3 (2%)	0 (0%)	1 (8%)	**4 (2%)**
Unexplained (all)	68 (51%)	19(61%)	4 (31%)	**91 (51%)**
Total:	**133 (75%)**	**31 (18%)**	**13 (7%)**	**177**

Table 58 cause of death for maternal age in late miscarriage (excludes 3 cases with no maternal age)

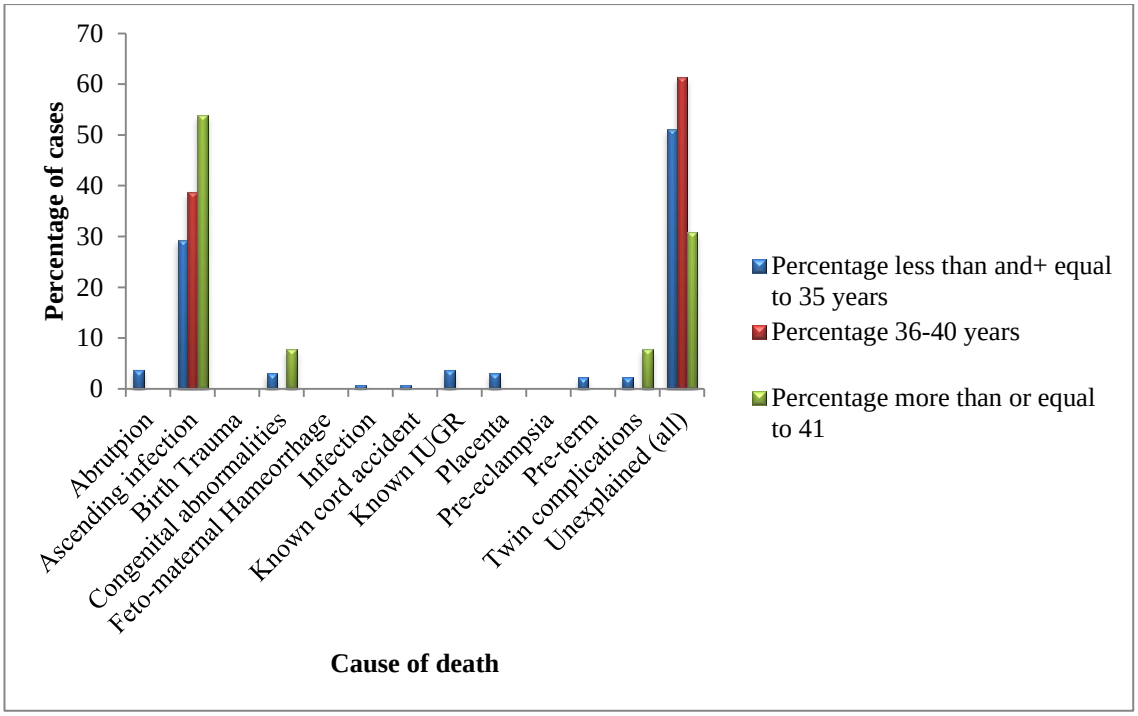


Figure 29 *Cause of death in late miscarriage by maternal age*

4.6.4 Maternal age and cause of death in cases of Stillbirth

Cause of death	Maternal age less than or equal to 35 years	Maternal age 36 -40 years	Maternal age more than or equal to 41 years	**Total:**
Abruption	20 (4%)	7 (7%)	1 (4%)	**28 (5%)**
Ascending infection	46 (9%)	11 (11%)	1 (4%)	**58 (9%)**
Birth Trauma	6 (1%)	0 (0%)	0 (0%)	**6 (1%)**
Congenital abnormalities	29 (6%)	3 (3%)	3 (12%)	**35 (6%)**
Feto-maternal Haemorrhage	4 (1%)	1(1%)	1 (4%)	**6 (1%)**
Infection	10 (2%)	1 (1%)	1 (4%)	**12 (2%)**
Known cord accident	2 (<1%)	0 (0%)	0 (0%)	**2 (<1%)**
Known IUGR	6 (1%)	3 (3%)	0 (0%)	**9 (1%)**
Placenta	36(7%)	7(7%)	6 (24%)	**49 (8%)**
Pre-eclampsia	14 (3%)	2 (2%)	0 (0%)	**16 (3%)**
Pre-term	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Twin complications	7 (1%)	2 (2%)	0 (0%)	**9 (1%)**
Unexplained (all)	314 (63%)	65 (64%)	12 (48%)	**391 (63%)**
Total:	**495 (78%)**	**102 (16%)**	**25 (4%)**	**622**

Table 59 Cause of death in stillbirth for maternal age (excludes 17 cases with no maternal age)

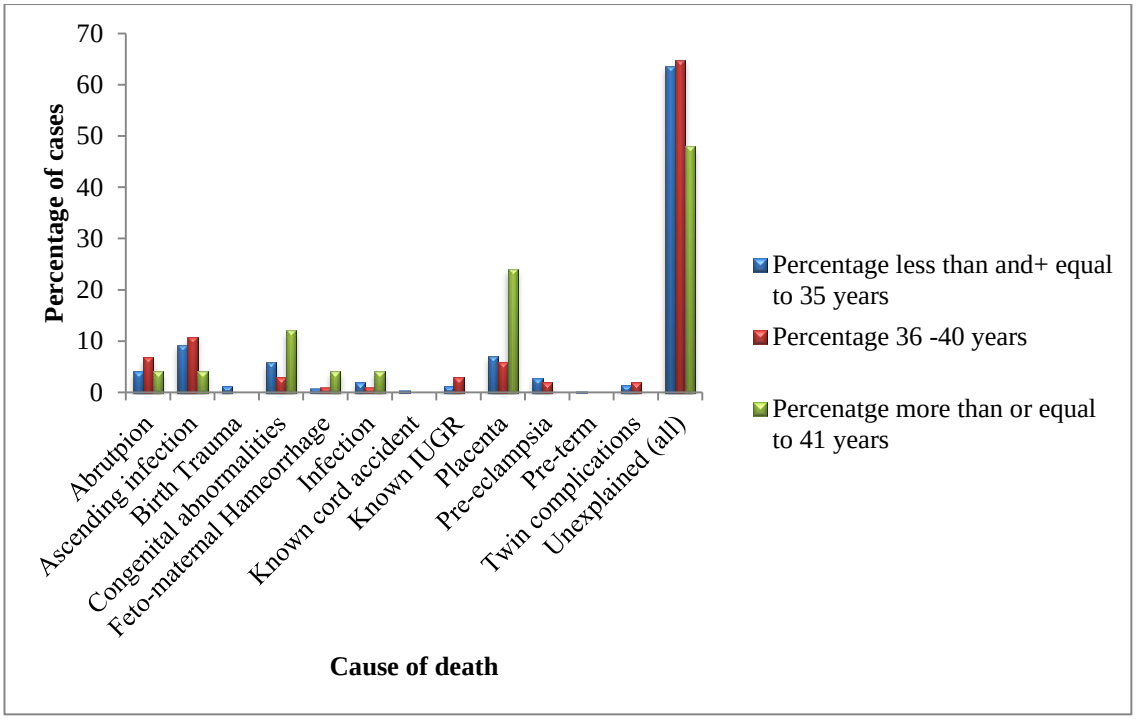


Figure 30 *Causes of death in stillbirth by maternal age*

4.7 Maternal Body Mass Index (BMI) and cause of death

4.7.1 Maternal BMI and cause of death in all cases

Cause of death	BMI Underweight	BMI Normal	BMI Overweight	BMI Obese	**Total:**
Abruption	0 (0%)	4 (3%)	4 (2%)	2 (1%)	**10 (2%)**
Ascending Infection	0 (0%)	30 (19%)	30 (19%)	25 (16%)	**84 (18%)**
Birth Trauma	0 (0%)	1 (1%)	3 (2%)	0 (0%)	**4 (1%)**
Congenital abnormalities	0 (0%)	9 (6%)	5 (3%)	5 (3%)	**19 (4%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	1 (1%)	0 (0%)	**1 (<1%)**
Infection	0 (0%)	1 (1%)	2 (1%)	6 (4%)	**9 (2%)**
Known cord accident	0 (0%)	0 (0%)	0 (0%)	1 (1%)	**1 (<1%)**
Known IUGR	0 (0%)	6 (4%)	2 (1%)	3 (2%)	**11 (2%)**
Placenta	0 (0%)	7 (6%)	8 (5%)	5 (3%)	**20 (4%)**
Pre-eclampsia	0 (0%)	4 (3%)	2 (1%)	2 (1%)	**8 (2%)**
Pre-term	0 (0%)	1 (1%)	0 (0%)	0 (0%)	**1 (<1%)**
Twin Complication	0 (0%)	1 (1%)	6 (4%)	4 (3%)	**11 (2%)**
Unexplained (all)	4 (100%)	90 (58%)	97 (60%)	96 (65%)	**287 (61%)**
Total:	**4 (1%)**	**156 (33%)**	**161 (35%)**	**149 (32%)**	**467**

Table 60 Cause of death by maternal BMI (all)

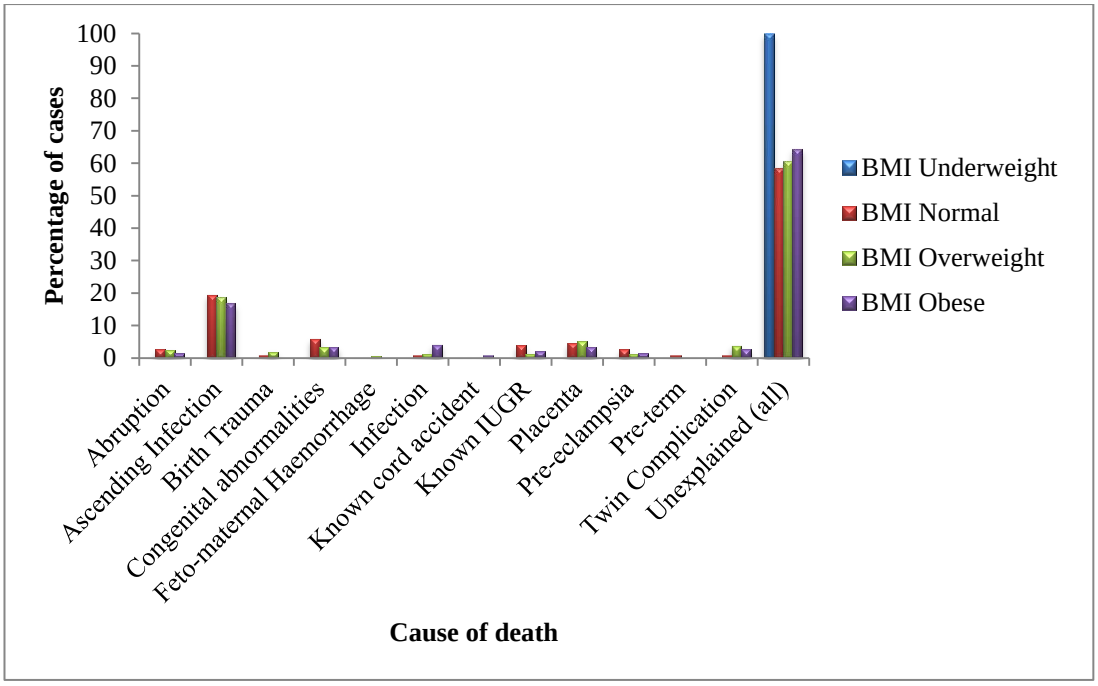


Figure 31 *Cause of death (all) by maternal BMI*

There was no significant difference in the proportion of unexplained deaths or cases of ascending infection in obese mothers in comparison to all other BMIs.

4.7.2 Maternal BMI and cause of death in cases of Early miscarriage

Cause of death	BMI Underweight	BMI Normal	BMI Overweight	BMI Obese	**Total:**
Abruption	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Ascending Infection	0 (0%)	14 (33%)	12 (29%)	6 (14%)	**32 (25%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	0 (0%)	2 (5%)	1 (2%)	2 (5%)	**5 (4%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known cord accident	0 (0%)	0 (0%)	0 (0%)	1 (2%)	**1 (1%)**
Known IUGR	0 (0%)	1 (2%)	0 (0%)	0 (0%)	**1 (1%)**
Placenta	0 (0%)	0 (0%)	1 (2%)	0 (0%)	**1 (1%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Pre-term	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Twin Complication	0 (0%)	0 (0%)	4 (10%)	4 (9%)	**8 (6%)**
Unexplained (all)	1 (100%)	25 (60%)	23 (56%)	30 (70%)	**79 (62%)**
Total:	**1 (1%)**	**42 (34%)**	**41 (32%)**	**43 (34%)**	**127**

Table 61 Cause of death for early miscarriages maternal BMI

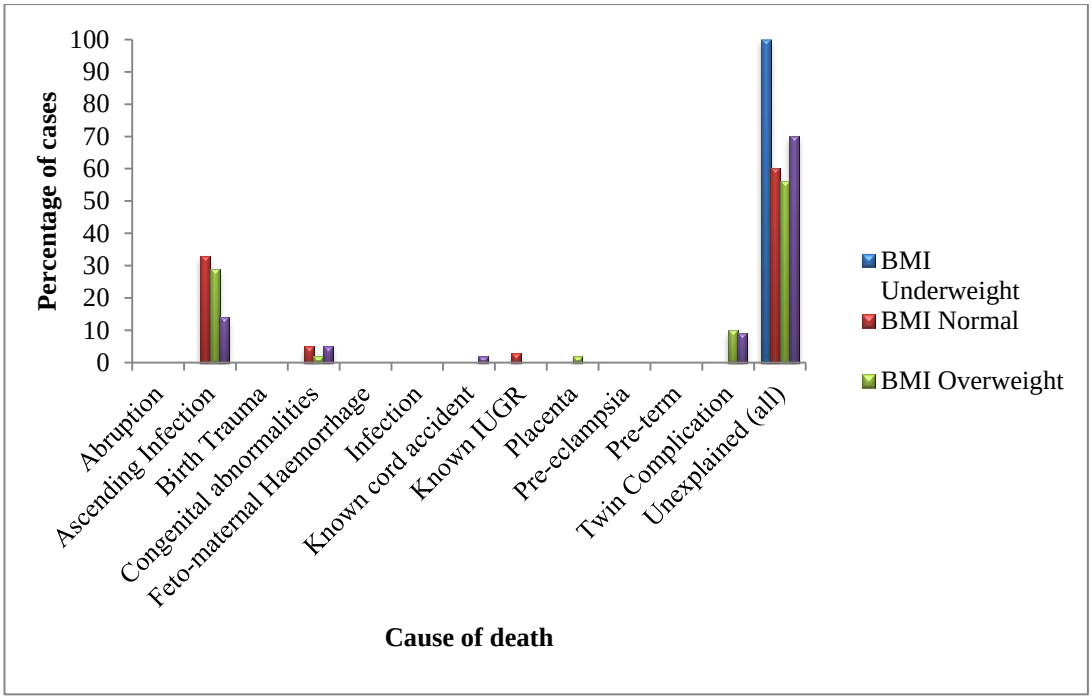


Figure 32 *Cause of death for early miscarriages with maternal BMI*

4.7.3 Maternal BMI and cause of death in cases of Late miscarriage

Cause of death	BMI Underweight	BMI Normal	BMI Overweight	BMI Obese	**Total:**
Abruption	0 (0%)	0(0%)	1 (3%)	0(0%)	**1 (1%)**
Ascending Infection	0 (0%)	7 (28%)	14 (41%)	9 (45%)	**29 (37%)**
Birth Trauma	0 (0%)	0(0%)	2 (5%)	0(0%)	**2 (3%)**
Congenital abnormalities	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Feto-maternal Haemorrhage	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Infection	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Known cord accident	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Known IUGR	0 (0%)	1 (4%)	1 (3%)	2 (10%)	**4 (5%)**
Placenta	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Pre-eclampsia	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Pre-term	0 (0%)	0(0%)	0(0%)	0(0%)	**0(0%)**
Twin Complication	0 (0%)	1 (4%)	0(0%)	0(0%)	**1 (1%)**
Unexplained (all)	0 (0%)	16 (64%)	16 (47%)	9 (45%)	**41 (52%)**
Total:	**0 (0%)**	**25 (32%)**	**34 (43%)**	**20 (25%)**	**79**

Table 62 Cause of death in late miscarriage by maternal BMI

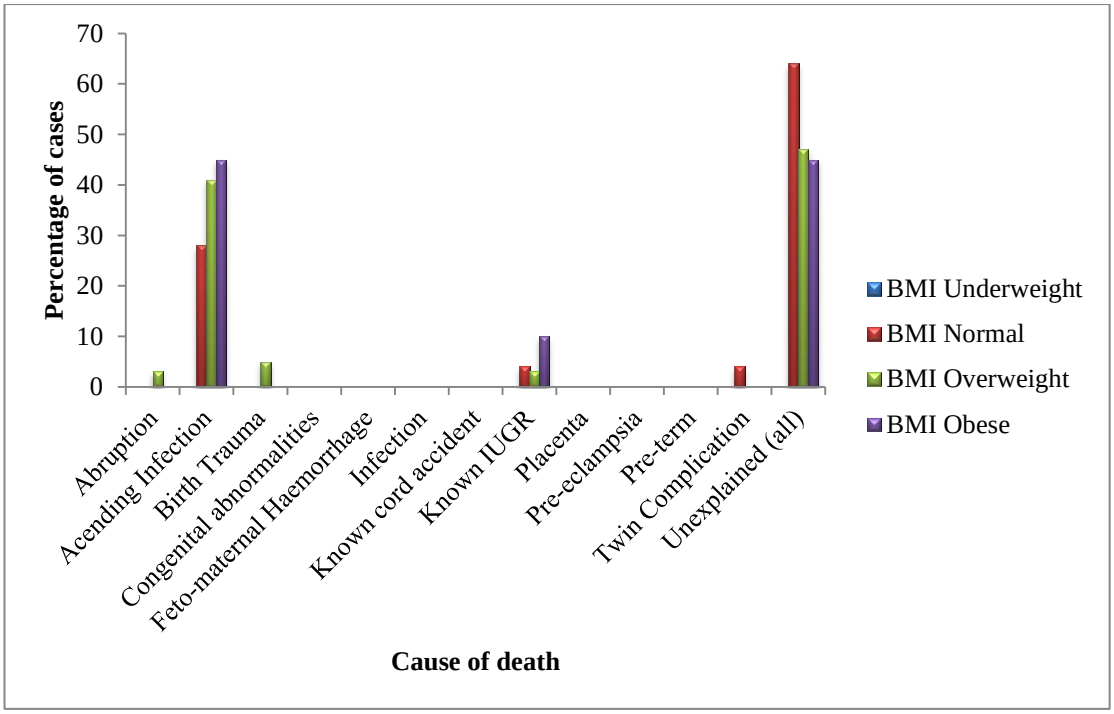


Figure 33 *Cause of death in late miscarriage for maternal BMI*

4.7.4 Maternal BMI and cause of death in cases of Stillbirth

Cause of death	BMI Underweight	BMI Normal	BMI Overweight	BMI Obese	**Total:**
Abruption	0 (0%)	4 (5%)	3 (3%)	2 (2%)	**9 (3%)**
Acending Infection	0 (0%)	9 (10%)	4 (5%)	10 (12%)	**23 (9%)**
Birth Trauma	0 (0%)	0 (0%)	2 (2%)	0 (0%)	**2 (1%)**
Congenital abnormalities	0 (0%)	7 (8%)	4 (5%)	3 (4%)	**14 (5%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	1 (1%)	0 (0%)	**1 (<1%)**
Infection	0 (0%)	1 (1%)	2 (2%)	6 (7%)	**9 (3%)**
Known cord accident	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known IUGR	0 (10%)	4 (5%)	1 (2%)	1 (1%)	**6 (2%)**
Placenta	0 (0%)	7 (8%)	7 (8%)	5 (6%)	**19 (4%)**
Pre-eclampsia	0 (0%)	4 (5%)	2 (2%)	2 (2%)	**8 (3%)**
Pre-term	0 (0%)	1 (1%)	0 (0%)	0 (0%)	**1 (<1%)**
Twin Complication	0 (0%)	0 (0%)	2 (2%)	0 (0%)	**2 (1%)**
Unexplained (all)	3 (100%)	49 (57%)	58 (67%)	57 (66%)	**167(64%)**
Total:	**3 (1%)**	**86 (33%)**	**86 (33%)**	**86 (33%)**	**261**

Table 63 Cause of death for stillbirth maternal BMI

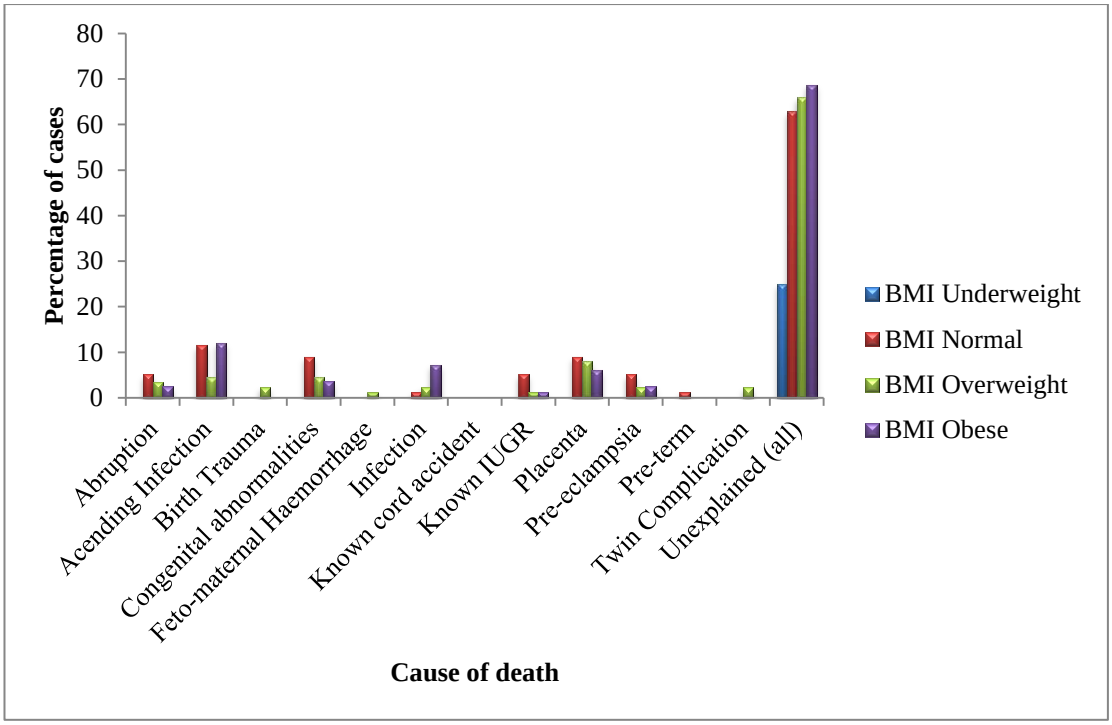


Figure 34 *Cause of death in stillbirth by maternal BMI*

4.8 Maternal Diabetes Mellitus and Cause of death

Cause of death	Early Miscarriage Any Diabetes Mellitus	Late miscarriage Any Diabetes Mellitus	Stillbirth Any Diabetes Mellitus	Total:
Abruption	0 (0%)	0 (0%)	0 (0%)	0 (0%
Acending Infection	3 (33%)	1 (14%)	4 (9%)	8 (13%)
Birth Trauma	0 (0%)	0 (0%)	1 (2%)	1 (2%)
Congenital abnormalities	0 (0%)	0 (0%)	1 (2%)	1 (2%)
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Infection	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Known cord accident	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Known IUGR	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Placenta	0 (0%)	0 (0%)	5 (11%)	5 (8%)
Pre-eclampsia	0 (0%)	0 (0%)	2 (4%)	2 (3%)
Pre-term	0 (0%)	0 (0%)	0 (%)	0 (0%)
Twin Complication	1 (11%)	1 (14%)	2 (4%)	4 (6%)
Unexplained (all)	5 (56%)	5 (71%)	32 (68%)	43 (68%)
Total:	**9 (14%)**	**7 (11%)**	**47 (75%)**	**63**

Table 64 Cause of death in all cases of maternal Diabetes Mellitus

Cause of death	Number of cases with DM of any kind	No DM	**Total:**
Abruption	0 (0%)	38 (4%)	**38 (4%)**
Acending Infection	8 (13%)	168 (17%)	**176 (17%)**
Birth Trauma	1 (2%)	6 (1%)	**7 (1%)**
Congenital abnormalities	1 (2%)	49 (5%)	**50 (5%)**
Feto-maternal Haemorrhage	0 (0%)	6 (1%)	**6 (1%)**
Infection	0 (0%)	13 (1%)	**13 (1%)**
Known cord accident	0 (0%)	4 (<1%)	**4 (<1%)**
Known IUGR	0 (0%)	17 (2%)	**17 (2%)**
Placenta	4 (6%)	52 (5%)	**56 (5%)**
Pre-eclampsia	2 (3%)	14 (1%)	**16 (2%)**
Pre-term	0 (0%)	4 (<1%)	**4 (<1%)**
Twin Complication	4 (6%)	17 (2%)	**21 (2%)**
Unexplained (all)	43 (68%)	613 (61%)	**656 (62%)**
Total:	**63**	**1001**	**1064**

Table 65 Cause of death in all cases with any maternal Diabetes Mellitus and no maternal Diabetes Mellitus

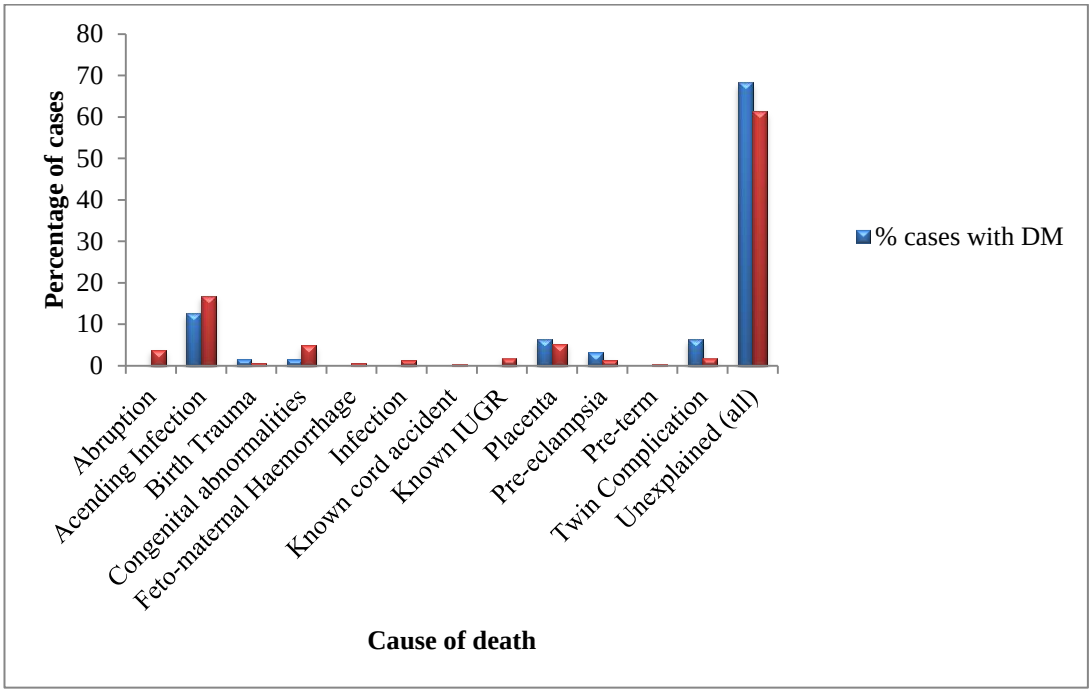


Figure 35 *Cause of death in all cases with any maternal Diabetes Mellitus and no maternal Diabetes Mellitus. Significantly greater proportion of twin complications in diabetic mothers compared to non-diabetic mothers (z=2.574, p= 0.01).*

There was a significantly greater proportion of twin complications in diabetic mothers compared to non-diabetic mothers (z=2.574, p= 0.01) of uncertain significance. No other significant differences were observed between diabetic and non-diabetic mothers.

4.9 Maternal Hypertension and Cause of death

Cause of death	Early miscarriage Hypertension	Late miscarriage hypertension	Stillbirth Hypertension	Total Hypertension
Abruption	0 (0%)	0 (0%)	6 (9%)	**6 (7%)**
Ascending Infection	3 (38%)	4 (44%)	1 (1%)	**8 (9%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	0 (0%)	1 (11%)	1 (1%)	**2 (2%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%)	2 (3%)	**2 2%)**
Known cord accident	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known IUGR	0 (0%)	0 (0%)	1 (1%)	**1 (1%)**
Placenta	0 (0%)	1 (11%)	10 (14%)	**11 (13%)**
Pre-eclampsia	0 (0%)	0 (0%)	14 (20%)	**14 (16%)**
Pre-term	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Twin Complication	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Unexplained (all)	5 (63%)	3 (33%)	34 (49%)	**42 (49%)**
Total:	**8 (9%)**	**9 (10%)**	**69 (80%)**	**86**

Table 66 Cause of death in those mothers with hypertension

Cause of death	Mothers with hypertension	Mothers with no hypertension	**Total:**
Abruption	6 (7%)	32 (3%)	**38 (4%)**
Ascending Infection	8 (9%)	168 (17%)	**176 (17%)**
Birth Trauma	0 (0%)	7 (1%)	**7 (1%)**
Congenital abnormalities	2 (2%)	48 (5%)	**50 (5%)**
Feto-maternal Haemorrhage	0 (0%)	6 (1%)	**6 (1%)**
Infection	2 (2%)	11 (1%)	**13 (1%)**
Known cord accident	0 (0%)	4 (<1%)	**4 (<1%)**
Known IUGR	1 (1%)	16 (2%)	**17 (2%)**
Placenta	11 (13%)	45 (5%)	**56 (5%)**
Pre-eclampsia	14 (16%)	2 (<1%)	**16 (2%)**
Pre-term	0 (0%)	4 (<1%)	**4 (<1%)**
Twin Complication	0 (0%)	21 (2%)	**21 (2%)**
Unexplained (all)	42 (49%)	614 (63%)	**656 (62%)**
Total:	**86**	**978**	**1064**

Table 67 Cause of death in those mothers with and without hypertension

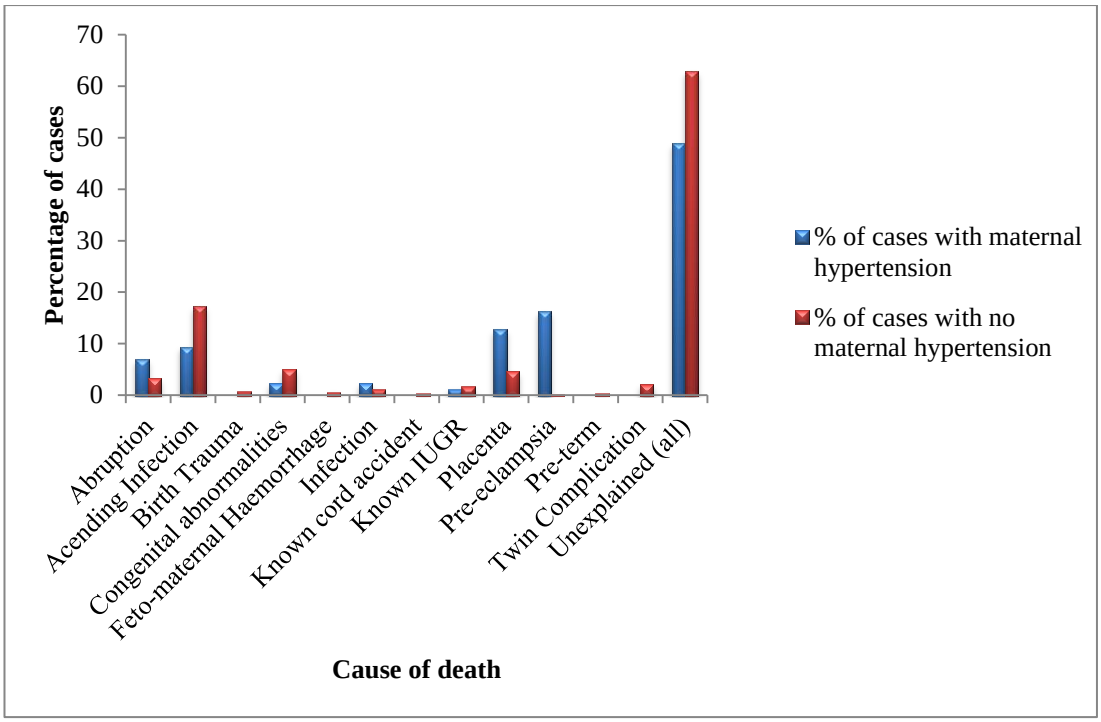


Figure 36 *All causes of death in those with and without maternal hypertension. Significantly greater proportion of unexplained deaths in non-hypertensive mothers (z=2.55, p = 0.0108). Significantly greater proportion of placental causes of death in hypertensive mothers (z=3.261, p=0.0011)*

There was a significantly greater proportion of placental causes of death in hypertensive mothers (z=3.261, p=0.0011) and a significantly greater proportion of unexplained deaths in non-hypertensive mothers (z=2.55, p=0.0108). No other significant differences were observed between the hypertensive and non-hypertensive mothers.

4.10 Fetal maceration and cause of death

4.10.1 Fetal maceration and cause of death in all cases

Cause of death	None	Mild	Moderate	Severe	**Total**
Abruption	23 (7%)	2 (2%)	2 (3%)	8 (2%)	**35 (4%)**
Ascending Infection	125 (39%)	22 (18%)	5 (8%)	16 (4%)	**168 (19%)**
Birth Trauma	7 (2%)	0 (0%)	0 (0%)	0 (0%)	**7 (1%)**
Congenital abnormalities	9 (3%)	2 (2%)	3 (5%)	22 (6%)	**36 (4%)**
Feto-maternal Haemorrhage	1 (<1%)	0 (0%)	0 (0%)	5 (1%)	**6 (1%)**
Infection	3 (1%)	2 (2%)	1 (2%)	4 (1%)	**10 (1%)**
Known cord accident	1 (<1%)	3 (3%)	0 (0%)	0 (0%)	**4 (<1%)**
Known IUGR	5 (2%)	3 (3%)	1 (2%)	5 (1%)	**14 (2%)**
Placenta	3 (1%)	7 (6%)	8 (14%)	28 (7%)	**46 (5%)**
Pre-eclampsia	3 (1%)	4 (3%)	1 (2%)	2 (1%)	**10 (1%)**
Pre-term	4 (1%)	0 (0%)	0 (0%)	0 (0%)	**4 (<1%)**
Twin Complication	12 (4%)	1 (1%)	0 (0%)	5 (1%)	**18 (2%)**
Unexplained (all)	128 (40%)	73 (61%)	38 (64%)	297 (76%)	**536 (60%)**
Total:	**324 (36%)**	**119 (13%)**	**59 (7%)**	**392 (44%)**	**894**

Table 68 Cause of death and fetal maceration.

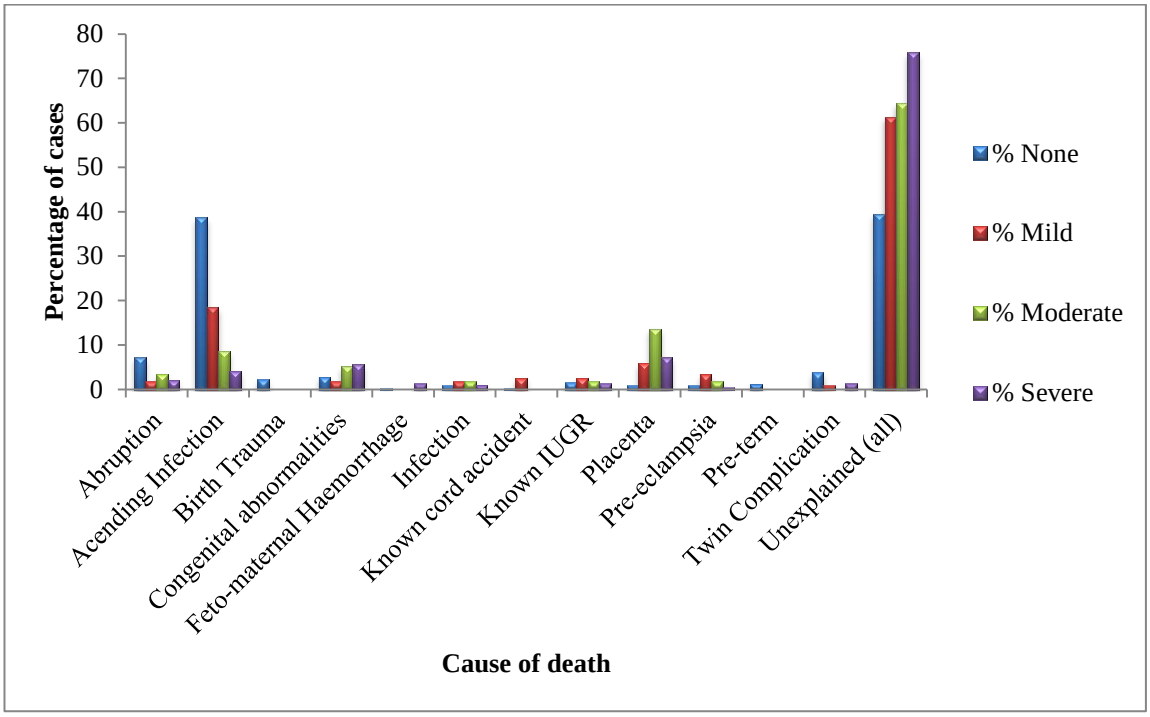


Figure 37 *Cause of death and fetal maceration*

Unexplained deaths were significantly more frequent in macerated cases ($z=9.408$, $p<0.0001$) and there is a stepwise increase in the proportion of unexplained deaths with increasing maceration.

If cases of ascending infection are excluded from maceration statistical tests, the association between unexplained deaths and maceration remains strongly significantly ($z=3.581$, $p=0.0003$)

4.10.2 Fetal maceration and cause of death in cases of Early Miscarriage

Cause of death	None	Mild	Moderate	Severe	**Total:**
Abruption	2 (2%)	0 (0%)	0 (0%)	1 (1%)	**3 (1%)**
Acending Infection	46 (48%)	3 (14%)	1 (33%)	6 (6%)	**56 (26%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	2 (2%)	0 (0%)	0 (0%)	6 (6%)	**8 (4%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known cord accident	0 (0%)	1 (5%)	0 (0%)	0 (0%)	**1 (<1%)**
Known IUGR	0 (0%)	0 (0%)	0 (0%)	4 (4%)	**4 (2%)**
Placenta	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1 (<1%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Pre-term	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Twin Complication	5 (5%)	1 (5%)	0 (0%)	2 (2%)	**8 (4%)**
Unexplained (all)	40 (42%)	17 (77%)	2 (67%)	75 (80%)	**134 (62%)**
Total:	**96 (45%)**	**22 (10%)**	**3 (1%)**	**94 (44%)**	**215**

Table 69 Cause of death in early miscarriage by fetal maceration (4 cases had no maceration given and 27 had "other" as their degree of maceration and were excluded).

4.10.3 Fetal maceration and cause of death in cases of Late miscarriage

Cause of death	None	Mild	Moderate	Severe	**Total:**
Abruption	4 (4%)	0 (0%)	0 (0%)	0 (0%)	**4 (3%)**
Acending Infection	48 (53%)	5 (42%)	0 (0%)	4 (10%)	**57 (38%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	0 (0%)	0 (0%)	0 (0%)	2 (5%)	**2 (1%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Infection	0 (0%)	0 (0%0	0 (0%)	0 (0%)	**0 (0%)**
Known cord accident	0 (0%)	1 (8%)	0 (0%)	0 (0%)	**1 (1%)**
Known IUGR	2 (2%)	2 (17%)	0 (0%)	0 (0%)	**4 (3%)**
Placenta	1 (1%)	0 (0%)	1 (17%)	2 (5%)	**4 (3%)**
Pre-eclampsia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Pre-term	3 (3%)	0 (0%)	0 (0%)	0 (0%)	**3 (2%)**
Twin Complication	4 (4%)	0 (0%)	0 (0%)	0 (0%)	**4 (3%)**
Unexplained (all)	29 (32%)	4 (33%)	5 (83%)	32 (80%)	**70 (47%)**
Total:	**91 (61%)**	**12 (8%)**	**6 (4%)**	**40 (27%)**	**149**

Table 70 Cause of death in late miscarriage by fetal maceration (4 cases not given maceration and 27 had "other" recorded and were excluded).

4.10.4 Fetal maceration and cause of death in cases of Stillbirth

Cause of death	None	Mild	Moderate	Severe	**Total:**
Abruption	17 (12%)	2 (2%)	2 (4%)	7 (3%)	**28 (5%)**
Acending Infection	31 (23%)	14 (16%)	4 (8%)	6 (2%)	**55 (10%)**
Birth Trauma	7 (5%)	0 (0%)	0 (0%)	0 (0%)	**7 (1%)**
Congenital abnormalities	7 (5%)	2 (2%)	3 (6%)	14 (5%)	**26 (5%)**
Feto-maternal Haemorrhage	1 (1%)	0 (0%)	0 (0%)	5 (2%)	**6 (1%)**
Infection	3 (2%)	2 (2%)	1 (2%)	4 (2%)	**10 (2%)**
Known cord accident	1 (1%)	1 (1%)	0 (0%)	0 (0%)	**2 (<1%)**
Known IUGR	3 (2%)	1 (1%)	1 (2%)	1 (<1%)	**6 (1%)**
Placenta	1 (1%)	7 (8%)	7 (14%)	28 (11%)	**43 (8%)**
Pre-eclampsia	3 (2%)	4 (4%)	1 (2%)	2 (1%)	**10 (2%)**
Pre-term	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1 (<1%)**
Twin Complication	3 (2%)	0 (0%)	0 (0%)	3 (1%)	**6 (1%)**
Unexplained (all)	59 (43%)	52 (61%)	31 (62%)	188 (73%)	**330(62%)**
Total:	**137 (26%)**	**85 (16%)**	**50 (9%)**	**258 (49%)**	**530**

Table 71 Cause of death in stillbirth by fetal maceration (14 cases not given maceration status and 95 had other description and were excluded)

4.10.5 Fetal Maceration in antepartum stillbirth

Cause of death	None	Mild	Moderate	Severe	**Total:**
Abruption	7 (18%)	2 (3%)	2 (4%)	6 (2%)	**17 (4%)**
Acending Infection	8 (20%)	11 (15%)	4 (8%)	5 (2%)	**28 (7%)**
Birth Trauma	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Congenital abnormalities	1 (3%)	2 (3%)	3 (6%)	14 (6%)	**20 (5%)**
Feto-maternal Haemorrhage	0 (0%)	0 (0%)	0 (0%)	5 (5%)	**5 (1%)**
Infection	0 (0%)	2 (3%)	1 (2%)	3 (1%)	**6 (1%)**
Known cord accident	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Known IUGR	2 (0%)	1 (1%)	1 (2%)	1 (<1%)	**5 (1%)**
Placenta	1 (3%)	7 (9%)	7 (14%)	28 (11%)	**43 (10%)**
Pre-eclampsia	2 (5%)	4 (5%)	1 (2%)	2 (1%)	**9 (2%)**
Pre-term	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Twin Complication	0 (0%)	0 (0%)	0 (0%)	2 (1%)	**2 (<1%)**
Unexplained (all)	19 (48%)	45 (61%)	30 (61%)	182 (73%)	**276 (67%)**
Total:	**40**	**74**	**49**	**248**	**411**

Table 72 Cause of death in antepartum stillbirth by fetal maceration (excluding those with "other maceration")

The more macerated the fetus in antepartum deaths, the greater the proportion of unexplained deaths; 7% of unexplained deaths are non-macerated and 62% of

unexplained deaths are severely macerated and the majority of antepartum deaths had severe maceration (60%).

4.10.6 Fetal Maceration in intrapartum stillbirth

Cause of death	None	Mild	Moderate	Severe	**Total:**
Abruption	10 (10%)	0 (0%)	0 (0%)	1 (8%)	**11 (9%)**
Ascending Infection	23 (24%)	3 (27%)	0 (0%)	1 (8%)	**27 (22%)**
Birth Trauma	7 (7%)	0 (0%)	0 (0%)	0 (0%)	**7 (6%)**
Congenital abnormalities	6 (6%)	0 (0%)	0 (0%)	0 (0%)	**6 (5%)**
Feto-maternal Haemorrhage	1 (1%)	0 (0%)	0 (0%)	0 (%)	**1 (1%)**
Infection	3 (3%)	0 (0%)	0(0%)	1 (0%)	**4 (3%)**
Known cord accident	1 (1%)	1 (0%)	0(0%)	0 (0%)	**2 (2%)**
Known IUGR	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1 (1%)**
Placenta	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Pre-eclampsia	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1 (1%)**
Pre-term	1 (1%)	0 (0%)	0 (0%)	0 (0%)	**1 (1%)**
Twin Complication	3 (3%)	0 (0%)	0 (0%)	1 (8%)	**4 (3%)**
Unexplained (all)	40 (41%)	7 (64%)	1 (100%)	8 (67%)	**56 (46%)**
Total:	**97**	**11**	**1**	**12**	**121**

Table 73 Cause of death in intrapartum stillbirth by fetal maceration (excluding those with "other maceration")

Antepartum stillbirths had a significantly greater proportion of unexplained deaths than intrapartum stillbirths ($z = 4.166$, $p<0.0001$) and the more macerated the fetus in antepartum deaths, the higher the proportion of unexplained deaths. This suggests that maceration may have an effect on the ability to give a cause of death in addition to the likelihood that intrapartum stillbirths will intrinsically have a more likely obvious explanation and be non macerated.

4.11 Postmortem interval and cause of death (overall)

Cause of death	1 - 4 days	5 - 8 days	9 -12 days	13-16 days	17+ days	**Total**
Abruption	6 (4%)	17 (4%)	12 (4%)	1 (1%)	1 (2%)	**37 (4%)**
Ascending Infection	24 (16%)	68 (15%)	46 (17%)	19 (18%)	11 (22%)	**168 (16%)**
Birth Trauma	2 (1%)	3 (1%)	6 (2%)	0 (0%)	0 (0%)	**11 (1%)**
Congenital abnormalities	7 (5%)	20 (4%)	12 (4%)	4 (4%)	1 (2%)	**44 (4%)**
Feto-maternal haemorrhage	1 (1%)	3 (1%)	0 (0%)	2 (2%)	0 (0%)	**6 (1%)**
Infection	5 (3%)	5 (1%)	3 (1%)	0 (0%)	0 (0%)	**13 (1%)**
Known cord accident	0 (0%)	2 (<1%)	1 (<1%)	0 (0%)	1 (2%)	**4 (<1%)**
Known IUGR	2 (1%)	8 (2%)	4 (1%)	1 (1%)	1 (2%)	**16 (2%)**
Placenta	5 (3%)	34 (7%)	9 (3%)	6 (6%)	1 (2%)	**53 (5%)**
Pre-eclampsia	1 (1%)	8 (2%)	3 (1%)	1 (1%)	2 (4%)	**15 (1%)**
Pre-term	0 (0%)	3 (1%)	0 (0%)	1 (1%)	0 (0%)	**4 (<1%)**
Twin complication	1 (1%)	9 (2%)	8 (3%)	2 (2%)	1 (2%)	**21 (2%)**
Unexplained (all)	96 (64%)	279 (61%)	171 (62%)	67 (64%)	32 (63%)	**647 (62%)**
Total:	**150 (14%)**	**459 (44%)**	**275 (26%)**	**104 (10%)**	**51 (5%)**	**1039**

Table 74 Cause of death by PM interval (25 cases excluded as no PM interval recorded.)

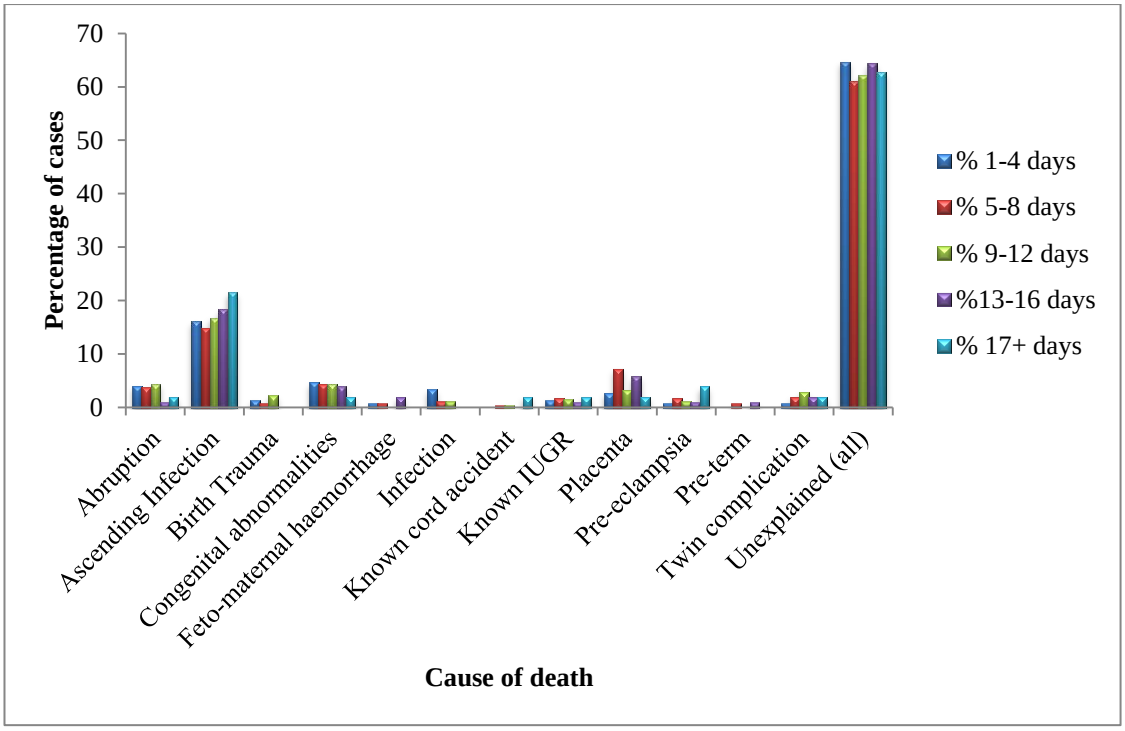


Figure 38 *Percentage of cases with each cause of death with different PM intervals*

There was no significant effect of PMI on cause of death determination.

4.12 ReCoDe classification

Calculations to determine birthweight centile (Chapter 5), have been used to determine the proportion of cases that are small for gestational age (SGA, <10th centile of the normal liveborn range) within the study population. These cases have been coded A7 – Fetal Growth Restriction together with those cases diagnosed with antenatal IUGR. (SGA cases could only be calculated for fetuses >23 weeks of gestation and therefore the total number of cases that could be classified using the ReCoDe system was 529).

Group A: Fetus
1. Lethal congenital anomaly
2. Infection
2.1 Chronic
2.2 Acute
3. Non-immune hydrops
4. Isoimmunisation
5. Fetomaternal haemorrhage
6. Twin-twin transfusion
7. Fetal growth restriction*

Group B: Umbilical cord
1. Prolapse
2. Constricting loop or knot†
3. Velamentous insertion
4. Other

Group C: Placenta
1. Abruption
2. Praevia
3. Vasa praevia
4. Other "placental insufficiency"‡
5. Other

Group D: Amniotic fluid
1. Chorioamnionitis
2. Oligohydramnios†
3. Polyhydramnios†
4. Other

Group E: Uterus
1. Rupture
2. Uterine anomalies
3. Other

Group F: Mother
1. Diabetes
2. Thyroid diseases
3. Essential hypertension
4. Hypertensive diseases in pregnancy
5. Lupus or antiphospholipid syndrome
6. Cholestasis
7. Drug misuse
8. Other

Group G: Intrapartum
1. Asphyxia
2. Birth trauma

Group H: Trauma
1. External
2. Iatrogenic

Group I: Unclassified
1. No relevant condition identified
2. No information available

* < 10th customised weight for gestational age centile.
†If severe enough to be considered relevant.

Figure 39 *Classification system according to relevant condition at death (ReCoDe)(5)*

ReCoDe Classification	Number of cases
A1	11 (2%)
A2	0 (0%)
A2.1	0 (0%)
A2.2	0 (0%)
A3	0 (0%)
A4	0 (0%)
A5	3 (1%)
A6	3 (1%)
A7	**193 (37%)**
B1	2 (<1%)
B2	0 (0%)
B3	0 (0%)
B4	10 (2%)
B5	0 (0%)
C1	11 (2%)
C2	0 (0%)
C3	0 (0%)
C4	10 (2%)
C5	24 (5%)
D1	36 (7%)
D2	0 (0%)
D3	0 (0%)
D4	0 (0%)
E1	0 (0%)
E2	0 (0%)
E3	2 (<1%)
F1	16 (3%)
F2	0 (0%)
F3	1 (<1%)
F4	6 (1%)
F5	0 (0%)
F6	5 (1%)
F7	0 (0%)
F8	9 (2%)
G1	0 (0%)
G2	3 (1%)
H1	0 (0%)
H2	0 (0%)
I1	**183 (35%)**
I2	0 (0%)
Total:	**528**

Table 75 ReCoDe classification of deaths.

Using this classification system, 37% of deaths were SGA and 35% were unexplained. The original ReCoDe paper classified 43% growth restricted and 15% as unexplained (5).

4.13 How often does the autopsy examination identify a specific cause of death?

Further analysis of the cause of death, using the objective criteria, demonstrated that 19% of the deaths could be classified based on the antenatal history, clinical events, antenatal ultrasound scan findings or external / imaging examination. Placental examination alone identified causes for a further 18%. Invasive autopsy examination, with associated organ sampling, only provided a specific cause of death in a minority (1%) of cases. The remainder of the deaths in the study population were unexplained and invasive autopsy provided no additional diagnostic information based on currently available ancillary investigations.

Of the 1% of cases in which a cause of death was determined at invasive autopsy;

- 55% were based on histology findings (the majority being lung histology- see histology chapter)
- 9% were based on microbiology findings,
- 27% were based on an internal, macroscopic assessment of organs.

Autopsy examination	Number of cases
Diagnosis determined by Histology	6 (55%)
Diagnosis determined by Microbiology	1 (9%)
Diagnosis determined by internal examination of organs	3 (27%)
Diagnosis determined by abnormalities noted within the blood	1 (9%)
Total:	**11**

Table 76 Cause of death diagnosed at autopsy using different methods

Of the 1,064 stillbirth and miscarriage autopsies reviewed, histological analysis of tissue provided a specific cause of death in only six (<1%) cases. Histological assessment of tissue at autopsy is reviewed in greater detail in Chapter 6.

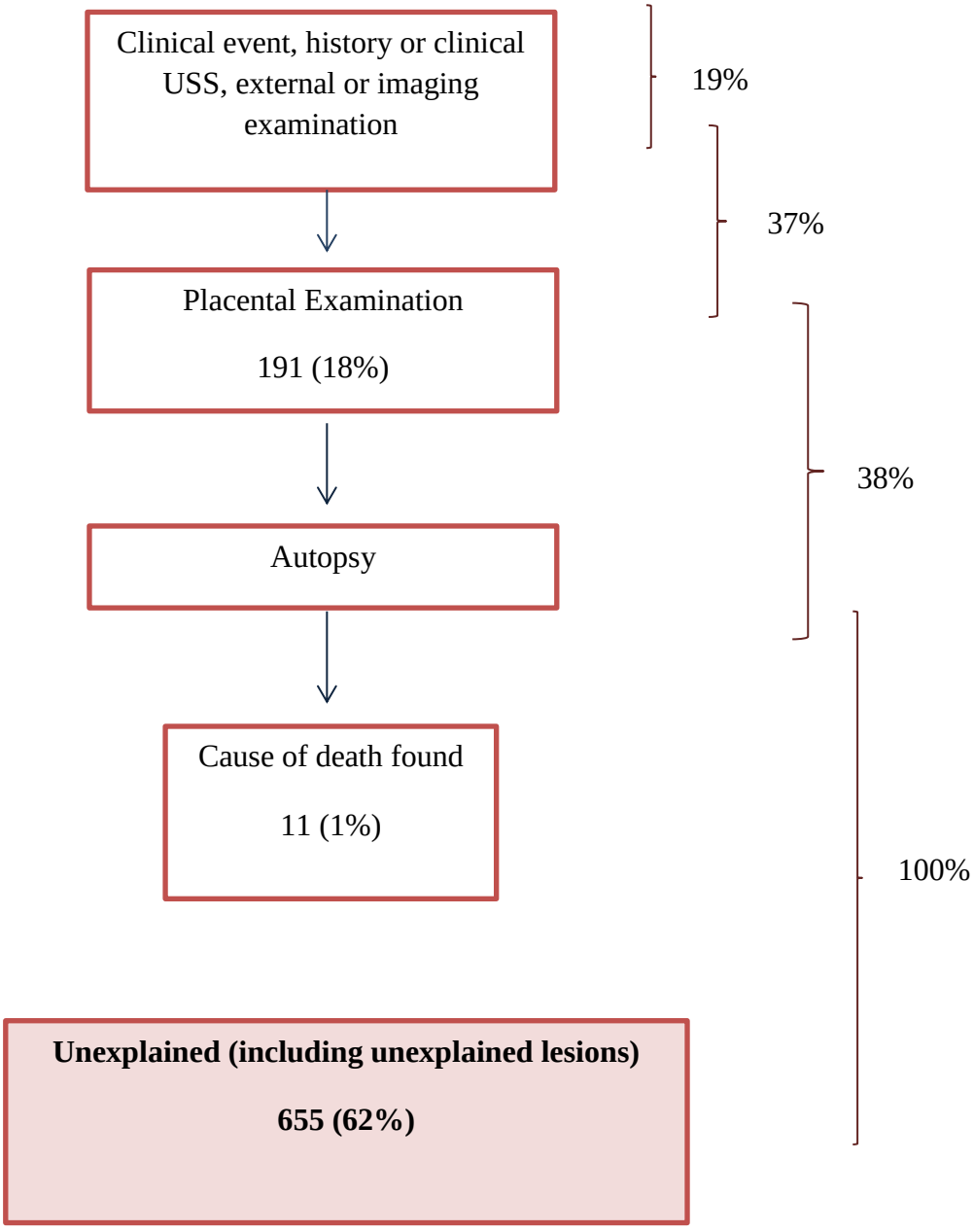


Figure 40 *proportion of cases that can be allocated a cause of death using either a clinical event, a good clinical history or ultrasound scan, those diagnosed after placental examination or autopsy and those with no diagnosis after all of the above.*

4.14 Discussion

Two thirds of the study population had an unexplained cause of death despite autopsy examination being performed: 27% of these deaths had no associated

clinical, fetal or placental lesions, being fully unexplained, whereas the remainder were unexplained but associated with known risk factors. These proportions are in keeping with other studies and highlight that depending on which classification system is used, 25-60% of deaths in stillbirth can be classified as unexplained based on whether associated conditions are deemed to be sufficient as a cause of death or simply a risk factor with the cause remaining unexplained (194). This has important consequences since it highlights that the great differences in cause of death assigned across studies, centres and classification systems is largely related to the interpretation of findings rather than any differences in findings themselves. Until objective laboratory criteria are available to determine or confirm mechanisms of death, these issues cannot be resolved. It should therefore be recognised that currently, a large proportion of the causes of death provided for such deaths are speculative, and differences in interpretation of features or findings accounts for differences in cause of death rates. This is important for interpretation of research data and for counselling parents.

The second most common cause of death overall was ascending infection, representing 17% of the total population, similar to that reported in other studies for high income countries (195-197). A large American study reported that the rate of chorioamnionitis, in both pre-term and term fetuses, has increased in all ethnicities over a 15 year period and white mothers continued to have a lower relative risk than all other ethnicities until 2009 (198). The present study confirms that Black and Asian mothers have significantly more cases of ascending infection than White mothers and that ascending infection is a major cause of miscarriage, particularly late miscarriage around 20-23 weeks of gestation.

White mothers had significantly more unexplained fetal deaths than other ethnicities, likely because of their lower rate of ascending infection. Mothers over the age of 40 had significantly more placental causes of death than mothers less than 40 years of age. There are no comparable published studies which examine in detail the specific maternal associations and causes of death in stillbirth.

Importantly, fetuses with more severe maceration changes, indicating a longer intrauterine interval between death and delivery (see chapter 5) were associated with a greater proportion of unexplained deaths. A stepwise increase in the proportion of unexplained deaths with worsening maceration was noted suggesting a likely association between the degree of maceration and the ability to identify a specific a cause of death at autopsy. A stepwise decrease in the number of cases of ascending infection with worsening maceration was also observed (as expected since ascending infection leads to onset of labour), which would account for some, but not all, of this difference; the relationship remains even if ascending infection cases are excluded. There was no effect on PMI and cause of death, demonstrating that delay between delivery and autopsy does not affect the likelihood of determining any specific cause of death, providing bodies are suitably refrigerated.

There were differences in cause of death categories according to gestational age at death. Placental abruption, maternal infection, placental abnormalities and unexplained lesions were significantly more frequent in later stillbirths than in earlier miscarriages whereas ascending infection and unexplained death with a previous history of fetal loss were significantly more frequent in miscarriages.

In order to allow comparison with published data, deaths in this study were also re-classified using ReCoDe. Using this classification (which includes risk factors as

well as causes of death), the proportion of unexplained deaths decreased and the highest proportion of deaths became attributed to growth restriction (37%), based solely on fetal biometry derived some time after the demise. (There are several major flaws with this approach, see Chapter 5 Fetal Growth Restriction Assessment). Nevertheless, using ReCoDe, 35% of stillbirths remained unexplained compared to a similar 26% in the current series being unexplained, unexplained (in the stillbirth sub-category). These findings demonstrate the difficulty in comparability between classification systems, even when the same population is being assessed, due to the arbitrary and subjective nature of most systems combined with difficulty in interpretation of the true significance of factors in any individual case. The original ReCoDe study reported a majority of growth restricted fetuses (43%), similar to that found in the current study (5). However the present study had more unexplained deaths than the ReCoDe study (26% vs 15.2%), largely since we applied strict criteria to assigning any findings as a cause of death (5).

Further analysis of the cause of death, using the study's objective criteria, found that almost 20% of the causes could have been identified from careful review of the antenatal history, clinical events, antenatal ultrasound scans or simple external examination or imaging of the fetus. Placental examination provided the cause of death for approximately 20% of additional cases. Full invasive autopsy examination itself only identified the cause of death in around 1% of cases, most remaining unexplained despite autopsy if placental and clinical history were non-contributory. Hence, published data, which include all aspects of the postmortem examination within the final "autopsy report", markedly overestimate the role of the invasive autopsy component itself in such cases. The findings suggest that:

1. The majority of stillbirths could be adequately investigated by detailed clinical review, external examination and/or imaging and placental examination, without the need for a full autopsy.
2. Only a minority (around 1%) of cases require an invasive autopsy to give a likely cause of death
3. Two thirds of stillbirth deaths cannot be given a definitive cause of death despite current antenatal, clinical and autopsy practise, and there is great variation in interpretation of many findings.
4. The major challenge remaining is therefore to develop novel methods of investigation to determine mechanisms of intrauterine death to reduce the proportion of "unexplained" cases.

It has been suggested that antenatally undiagnosed growth restriction is one of the main causes of death in stillbirth; Chapter 5 discusses this in detail (5). The present data estimate that only 10-20% of stillbirths are likely to be genuinely growth restricted and improving antenatal care to detect IUGR may reduce some otherwise unexplained deaths, but will not change the fact that the underlying mechanism of stillbirth remains unknown in most cases and only a minority are given a definite and accurate cause of death after autopsy. It is clear from these results that autopsy practise must evolve. There must be acceptance that areas of uncertainty exist and the usefulness of current autopsy techniques, with an aim to develop new methods of investigation with improved objectivity and efficacy.

5. Intrauterine Growth Restriction and Small for Gestational Age Fetuses

5.0 Background

5.1 Chapter Aims

5.2 Methods

- 5.2.1 Proportion of SGA cases

5.3 Results

- 5.3.1 Frequency of antenatally diagnosed FGR from the clinical history
- 5.3.2 Autopsy findings
- 5.3.3 Maternal obesity and SGA
- 5.3.4 Maternal ethnicity and SGA
- 5.3.5 Maternal Diabetes Mellitus and SGA
- 5.3.6 Maternal hypertension and SGA
- 5.3.7 Maternal age and SGA
- 5.3.8 Effects of fetal maceration
- 5.3.9 Effect of intrauterine and postmortem intervals on fetal weight and implications for SGA diagnosis at autopsy

5.4 Discussion

5.0 Background

Depending on which classification system is used, 15-60% of stillbirths remain unexplained, despite autopsy examinations being undertaken by trained professionals (4, 5). Much of this variation is related to the variable attribution of growth restriction to the cause of death.

Fetal growth restriction (FGR) implies a pathological restriction of the genetic growth potential whereas small for gestational age (SGA) simply describes a fetus whose biometry falls below an arbitrary centile, usually the 10^{th}, of the expected normal range for gestational age (199, 200). Not all FGR fetuses are SGA and not all SGA fetuses are FGR. Fetal birthweight is not only affected by pathological abnormalities but also physiological differences such as parental ethnicity and height as well as maternal parity (199). Indeed, 50-70% of SGA fetuses (based on standard charts) are likely to be constitutionally small, with fetal growth appropriate for maternal size and ethnicity (200). Customised growth charts have therefore been developed to reduce such effects and are recommended by the Royal College of Obstetricians and Gynaecologists in their 2014 Green Top Guideline (199, 201). Despite this, distinguishing incidental SGA from pathological FGR remains a challenge both antenatally and in particular postmortem.

FGR has been suggested as the single largest population attributable risk for stillbirth which could be significantly ameliorated by antenatal recognition (61). Studies from the 1990's report antenatal detection rates for fetuses delivered SGA of only 15-24% (84, 202). Using customised growth charts detection rates appear improved, to around 30%, suggesting that increased surveillance for SGA increases its detection (52, 61).

The Confidential Enquiry into Stillbirths with Fetal Growth Restriction reported an association between stillbirths with FGR and substandard care with such deaths considered potentially avoidable (203). The rate of stillbirth (per 1000 births) can vary from an average of 4.2, down to 2.4 without FGR; 9.7 when FGR is detected antenatally; and up to 19.8 when FGR is present but not detected during the antenatal period (52).

5.1 Chapter aims

This chapter aims to investigate the frequency of SGA and FGR in a large series of stillborn fetuses, highlighting maternal associations, specific causes of fetal death and the role that secondary changes such as fetal maceration, intrauterine interval and postmortem interval may have in the accuracy of diagnosing SGA and FGR at autopsy. Specifically, the chapter will cover:

1. The proportion of stillborn fetuses found to be SGA at autopsy.
2. The causes of death in cases of SGA and FGR stillborn fetuses.
3. Maternal demographic associations of SGA stillborn fetuses
4. The effects of fetal maceration and intrauterine/postmortem interval on fetal weight and SGA estimation postmortem.

5.2 Method

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriages from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Data was analysed through queries and statistical tests run using Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Statistical test results can be viewed in detail in Appendix 3.

5.2.1 Proportion of SGA

Neonatal growth charts published by the World Health Organisation (WHO) were used to determine normal expected weight centiles for fetuses born after 23 weeks' gestation. (Males and females were evaluated separately since infant sex has a marked effect on birthweight (204)). Individualised growth charts could not be used for this study due to its retrospective nature and the lack of detailed maternal demographic information required from archival postmortem reports. Nevertheless, since all comparisons are between groups, the findings and intergroup differences remain valid. These data were used to calculate the gestational age corrected expected birthweights and delta values for each case (the number of standard deviations (SDs) by which the observed birthweight differed from the expected 50th centile weight from the normal range.)

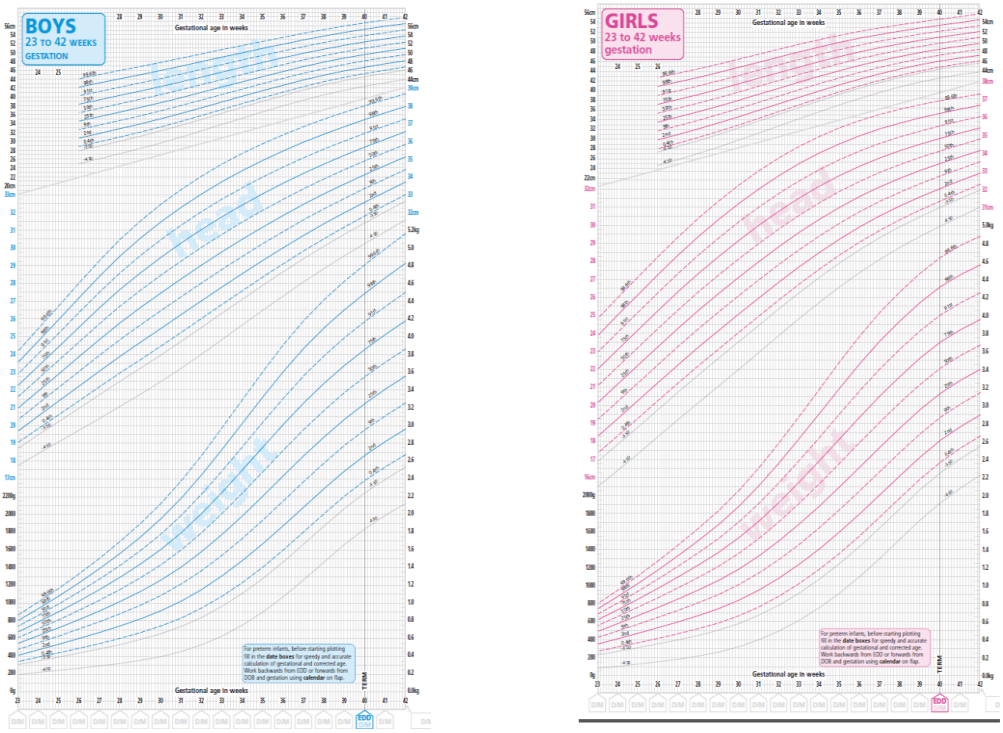


Figure 40 *WHO neonatal growth charts used as controls (Permission to reproduce this figure has been granted by RCPCH) (205)*

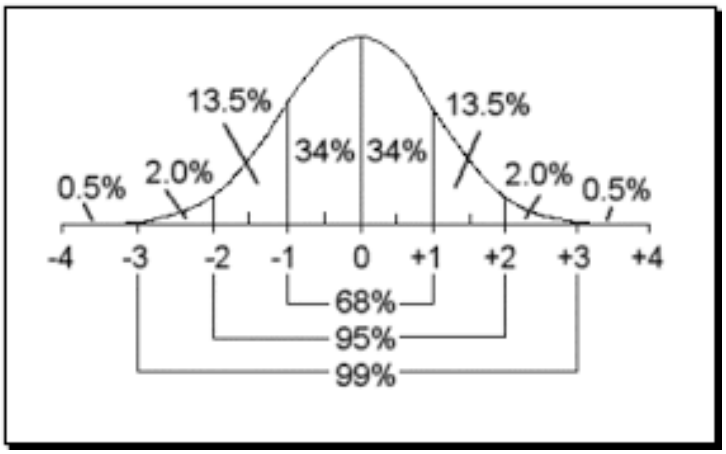


Figure 41 *Normal distribution curve (206)*

To calculate which fetuses were below the 10th centile in the study population the following calculation was used:

$$\frac{\text{(Observed weight (study birth weight) – Expected weight (WHO 50}^{th}\text{ centile for same gestation))}}{\text{SD in grams for gestation (calculated from WHO charts)}}$$

The resulting delta value corrects for gestational age and fetal sex effects and therefore allows valid comparison between groups regardless of the gestational age distributions. Cases with a delta value below -1.375 (approximating the 10th centile of the normal range) were denoted as SGA (see appendix 5).

5.3 Results

5.3.1 Frequency of antenatally diagnosed FGR from the clinical history

Of the total population, 43 (4%) cases had an antenatal diagnosis of Intrauterine Growth Restriction (IUGR) (*Table 77*), including 35 cases >23 weeks (35 of 674; 5%). 30 had delta birthweights available; 26 (87%) were SGA as well as being diagnosed with antenatal IUGR

25 of the total 43 cases (58%) had additional or other specific classification identified as the cause of death (*Table 78.*). The most common finding in this category was abnormality of the placenta such as infarction, followed by clinically diagnosed pre-eclampsia.

	Specific classification of death following autopsy	No other cause of death found at autopsy	Total:	Total study population
Known IUGR from antenatal History:	25 (58%)	18 (42%)	43 (4%)	1064

Table 77 Cause of death in known cases of IUGR. 35 cases out of 42 were >23 weeks gestation

Specific classification of death following autopsy in cases of antenatally detected IUGR	Number of cases
Placental abruption	3 (7%)
Ascending genital tract infection	1 (2%)
Congenital abnormalities	4 (9%)
Feto-maternal haemorrhage	1 (2%)
Placental malperfusion lesions	9 (21%)
Pre-eclampsia	7 (16%)
No specific additional found (therefore known IUGR given as COD in study)	18 (42%)
Total:	**43**

Table 78 Cause of death in cases of known antenatal IUGR

In the 58% of cases that had a specific identifiable cause of death at autopsy, this was used as the final cause of death. Where no other specific cause of death was identified, the case was classified as Known IUGR.

5.3.2 Autopsy findings

There were 533 stillbirths with a recorded birthweight delivered at greater than 23 weeks gestation. Of these, 192 (36%) had birthweights below the 10th centile and were thus SGA based on WHO charts for normal livebirths (*Table 79)*.

Male	Female
107 (56%) cases SGA(38% of all males)	85 (44%) cases SGA(34% of all females)

Table 79 Proportion of male and female stillbirths>23 weeks gestation that were SGA

Cause of death at autopsy in SGA cases

Cause of death	Number of cases
Abruption	11 (6%)
Ascending Infection	12 (6%)
Congenital abnormalities	17 (9%)
Feto-maternal Haemorrhage	2 (1%)
Infection	2 (1%)
Known IUGR	11 (6%)
Placenta	36 (19%)
Pre-term	0 (0%)
Pre-eclampsia	7 (4%)
Twin complications	4 (2%)
Unexplained	90 (47%)
Total:	**192**

Table 80 Causes of death in cases of SGA

Nearly half of cases of SGA (47%) had no specific identifiable cause of death (*Table 80*), whilst the most common specific category in this group was placental abnormalities (19%), typical of pathological FGR and uteroplacental malperfusion. These findings are similar to those observed in cases with antenatally detected IUGR. When comparing causes of death in non SGA and SGA fetuses, congenital abnormalities and placental causes of death were more frequently observed in SGA fetuses ($z= 2.796$ $p=0.0052$ and $z=5.897$, $p<0.0001$), however, unexplained deaths were more frequently observed in non SGA fetuses ($z= 5.582$, $p<0.0001$).

Cause of death	Non SGA	SGA
Abruption	12 (4%)	11(6%)
Ascending Infection	38 (11%)	12 (6%)
Birth Trauma	3 (1%)	0 (0%)
Congenital Abnormalities	11 (3%)	17 (9%)
Fetomaternal Haemorrhage	3 (1%)	2 (1%)
Infection	6 (2%)	2 (1%)
Known IUGR	0 (0%)	11 (6%)
Known cord accident	2 (1%)	0 (0%)
Placenta	12 (4%)	36 (19%)
Pre-eclampsia	6 (2%)	7 (4%)
Pre-term	2 (1%)	0 (0%)
Twin complication	3 (1%)	4 (2%)
Unexplained	243 (71%)	90 (47%)
Total:	**341**	**192**

Table 81 Causes of death in cases of Non SGA and SGA: congenital abnormalities and placental causes of death more frequent in SGA (z= 2.796 p=0.0052 and z=5.897, p<0.0001); unexplained deaths more frequent in non SGA (z= 5.582, p<0.0001).

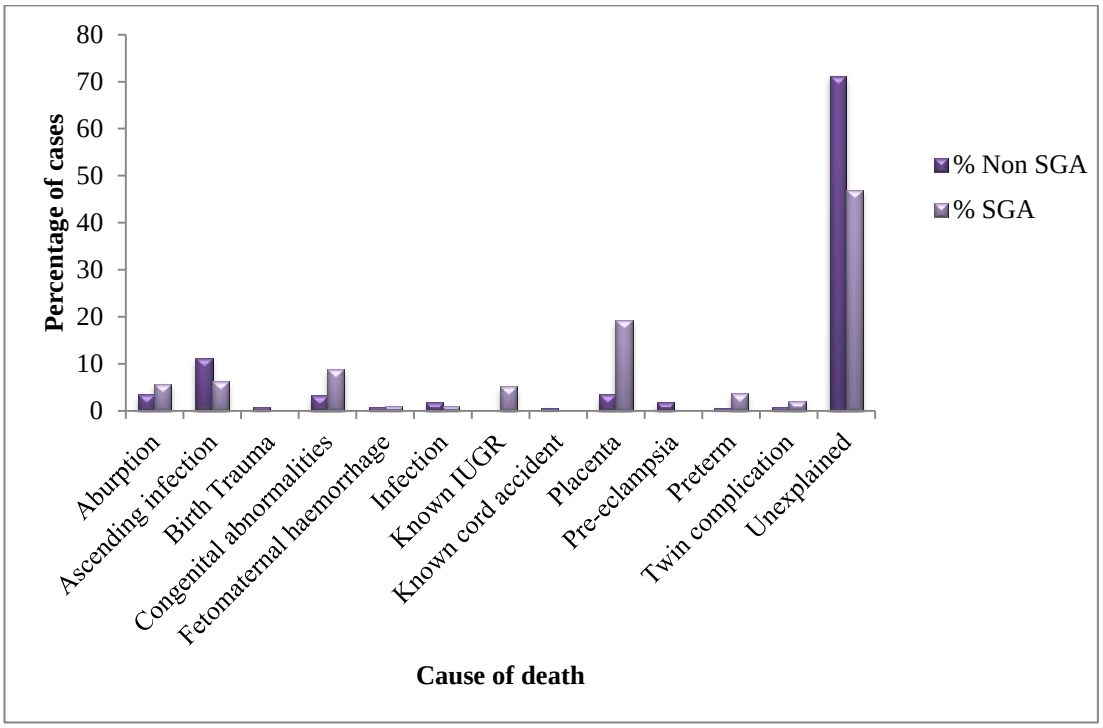


Figure 42 *Causes of death in cases of Non SGA and SGA: congenital abnormalities and placental causes of death more frequent in SGA (z=2.79, p=0.052 and z=5.897, p<0.0001); unexplained deaths more frequent in non SGA (z= 5.582, p<0.0001).*

Proportion of unexplained deaths with SGA

In the total study population there were 611 cases with an unexplained (all) cause of death, of whom 411 (67%) were stillbirths >23 weeks. 335 cases had a birthweight provided and therefore could be used in the SGA calculations. Of these unexplained deaths, 90 cases (27%) were SGA based on birthweight.

5.3.3 Maternal obesity and SGA

84 cases in the SGA population had a recorded maternal Body Mass Index (BMI). The majority of mothers had a normal BMI (significantly overrepresented in the SGA group z = 2.029, p=0.0425), and there was a significant overrepresentation of underweight mothers in the SGA group (*Table 82*) (p=0.02).

Maternal BMI	Non SGA	SGA
Underweight	0 (0%)	3 (4%)
Normal	43 (35%)	36 (42%)
Overweight	53 (34%)	22 (26%)
Obese	51(31%)	24 (28%)
Total	**147**	**85**

Table 82 Maternal BMI in cases of SGA and the remainder of the study population. There were significantly more underweight mothers in the SGA population (z= 2.293, p= 0.02)

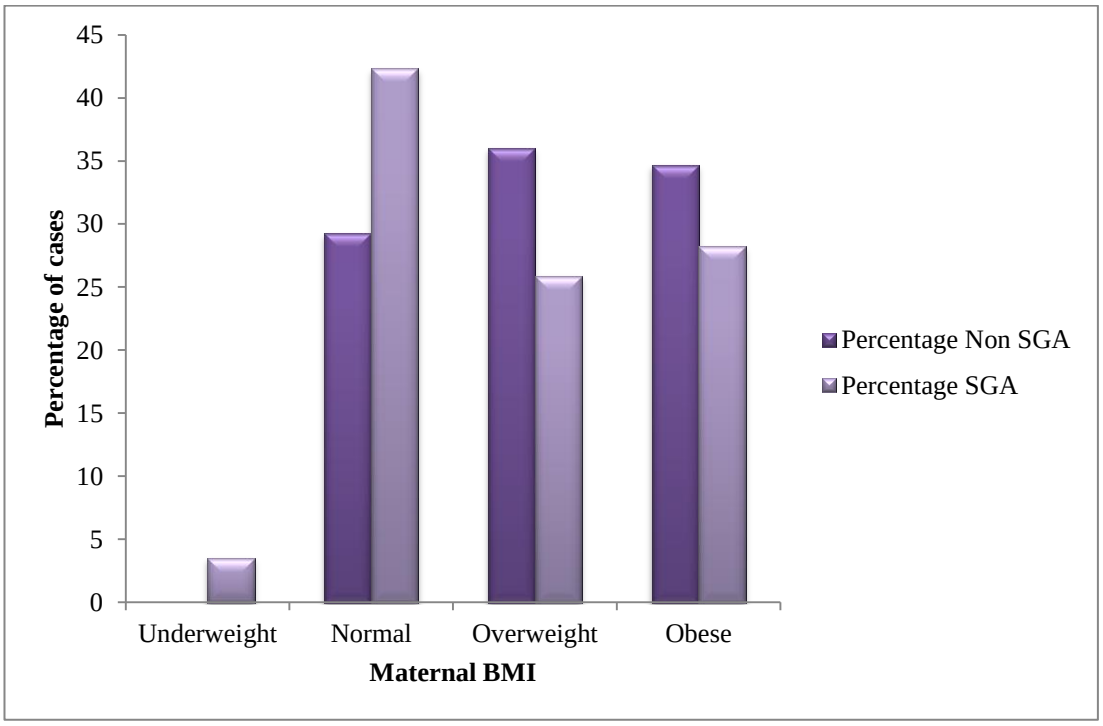


Figure 43 *Maternal BMI in cases of SGA and Non SGA. Normal weight and underweight mothers were significantly overrepresented in the SGA group (z = 2.029, p = 0.0425 and z=2.293, p= 0.02)*

5.3.4 Maternal ethnicity and SGA

154 SGA cases had a recorded maternal ethnicity. There were no significant differences between the two groups.

Maternal Ethnicity	Non SGA	SGA
White	174 (67%)	105 (68%)
Mixed	9 (3%)	2 (1%)
Asian	18 (7%)	15 (10%)
Black	57 (22%)	32 (21%)
Total	**258**	**154**

Table 83 Maternal ethnicity in cases of non SGA and SGA.

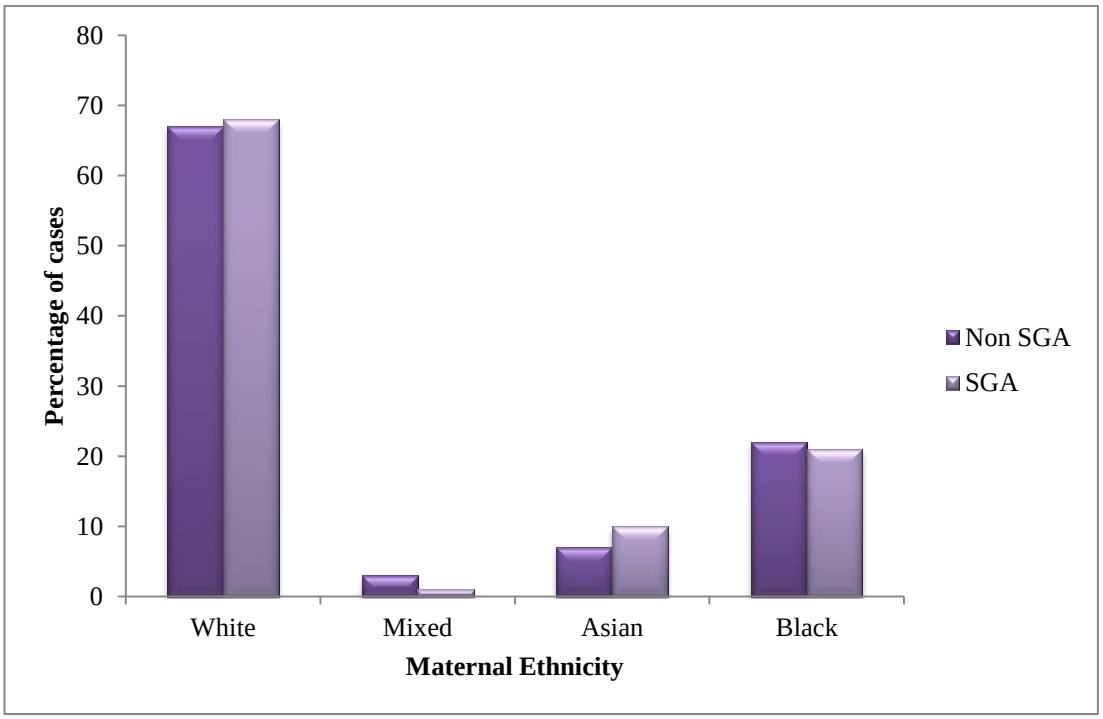


Figure 44 *Maternal ethnicity in non SGA and SGA cases.*

5.3.5 Maternal DM and SGA

11 cases in the SGA population had mothers with some form of diabetes mellitus. There was no significant difference in the proportion of mothers with gestational diabetes versus other forms of diabetes mellitus in the SGA group when compared to

the non SGA group (*Table* 85). There was also no significant difference in the number of mothers with any form of diabetes mellitus in the SGA population compared to the remainder of the study population.

Maternal Diabetes	Number of cases
Diabetes Mellitus	**5 (45%)**
On Insulin	2
No Insulin	1
No further information	2
Gestational DM	**6 (55%)**
Total:	11

Table 84 Maternal Diabetes Mellitus in cases of SGA

Maternal Diabetes Mellitus	Non SGA	SGA
Diabetes Mellitus	11 (38%)	5 (45%)
Gestational Diabetes	18 (62%)	6 (54%)
Total:	**29**	**11**

Table 85 Maternal Diabetes mellitus in cases of SGA and the remainder of the study population. No significant differences were found.

5.3.6 Maternal hypertension and SGA

24 (13%) SGA fetuses had mothers with some form of hypertension, the majority of which (42%) was pre-eclampsia (*Table 86).* There were no statistically significant differences between the two groups in the total number of cases with or without any form of hypertension, or between those with different types of hypertension.

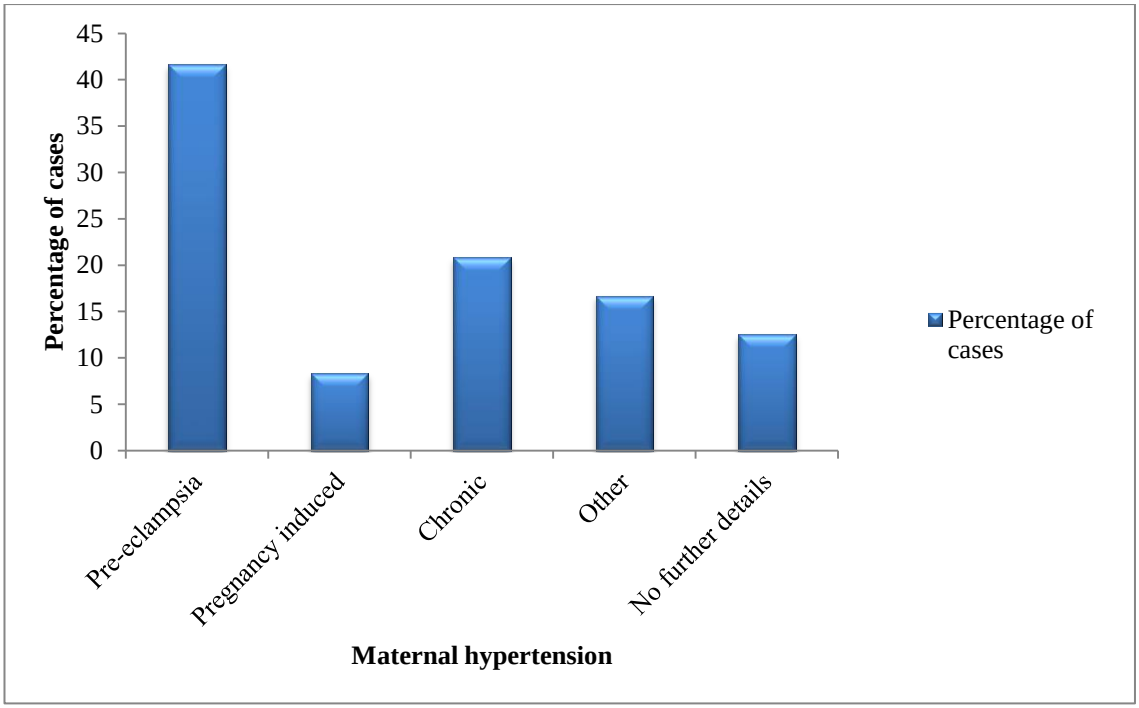


Figure 45 *Maternal hypertension in cases of SGA*

Maternal Hypertension	Non SGA	SGA
Pre-eclampsia	11 (32%)	10 (59%)
Pregnancy induced	12 (35%)	2 (12%)
Chronic	11 (32%)	5 (29%)
Total	**34**	**17**

Table 86 Maternal hypertension in cases of SGA and the remainder of the study population..

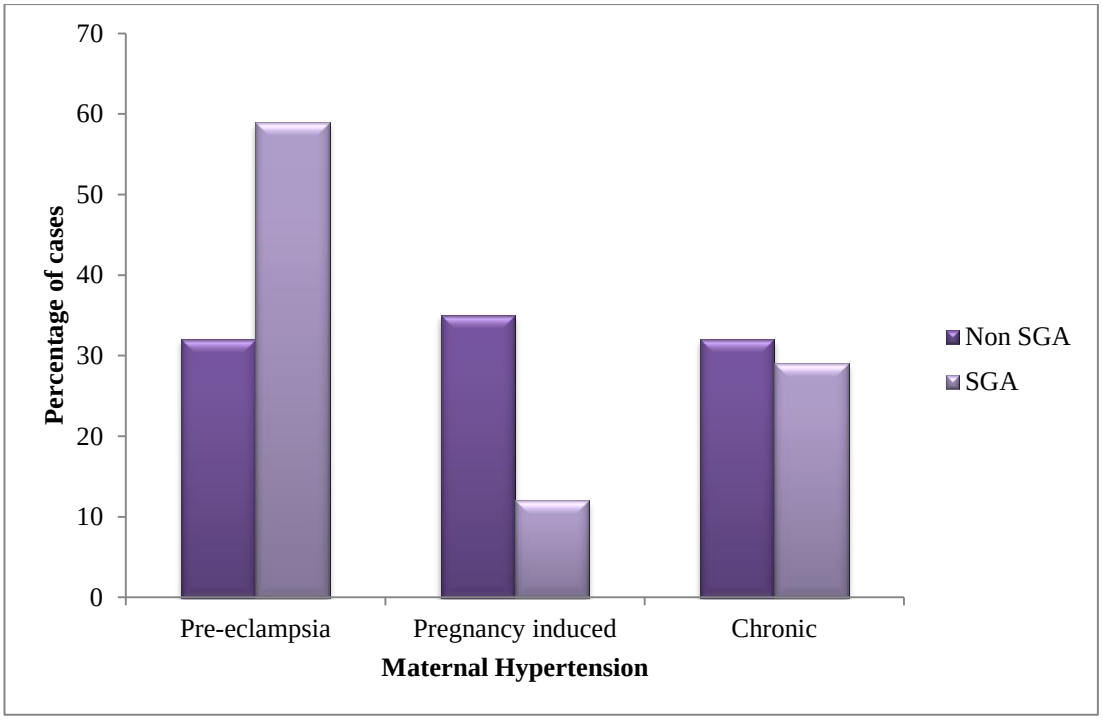


Figure 46 *Maternal hypertension in cases of SGA and the remainder of the study population.*

5.3.7 Maternal age and SGA

190 cases of SGA fetuses had a recorded maternal age (*Table 87 and Figure 47*). There was no significant difference between the two groups in mothers less than 35 years and greater than or equal to 35 years.

Maternal Age (years)	Non SGA Cases	SGA Cases
<19	20 (6%)	6 (3%)
20-24	39 (12%)	31 (16%)
25-29	80 (24%)	50 (26%)
30-34	114 (34%)	55 (29%)
35-39	62 (18%)	34 (18%)
40-44	23 (7%)	12 (6%)
>45	0 (0%)	2 (1%)
Total	**338**	**190**

Table 87 Maternal age distribution in cases of SGA and the remainder of the study population.

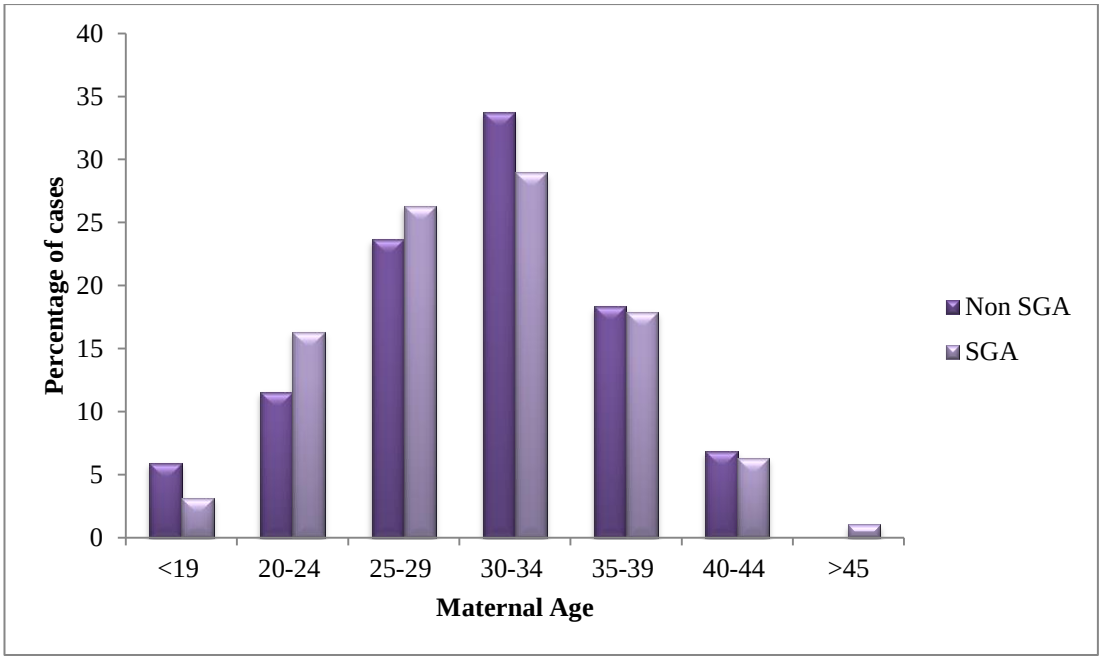


Figure 47 *Comparison of maternal age in the SGA population and the remainder of the study population.*

5.3.8 Effects of fetal maceration

86% of stillborn SGA fetuses had some degree of maceration noted at autopsy (*Table* 88), compared to 73% of non-SGA stillborn fetuses. Out of 415 cases with maceration, 28 (7%) were SGA with mild maceration and 100 (24%) were SGA with moderate or severe maceration.

Fetal Maceration	Number of cases
None	27 (14%)
Mild	28 (15%)
Moderate	12 (6%)
Severe	88 (46%)
Other description	35 (18%)
Total:	**190**
Not given	2

Table 88 Fetal maceration in cases of SGA

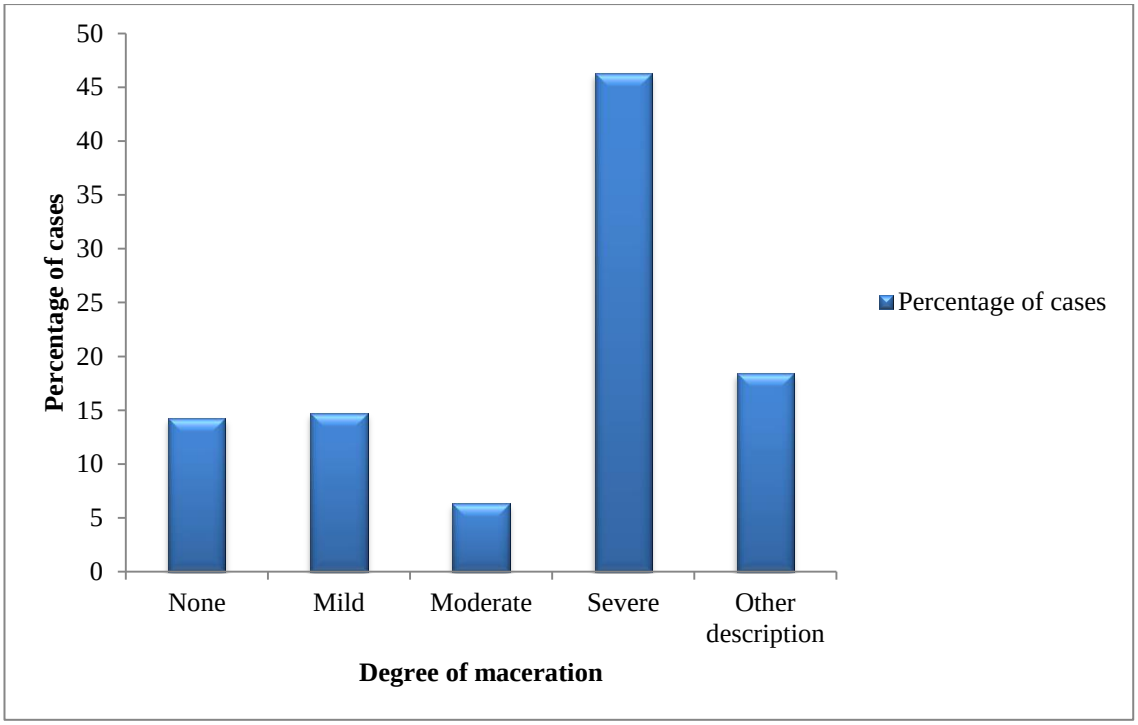


Figure 48 *Fetal maceration in cases of SGA*

A significantly greater proportion of SGA fetuses were macerated compared to the non SGA population (z=2.571 p=0.0102).

Degree of maceration	Non SGA	SGA
None	79 (24%)	27 (14%)
Mild	47 (14%)	28 (15%)
Moderate	31 (9%)	12 (6%)
Severe	128 (38%)	88 (46%)
Other	50 (15%)	35 (18%)
Total:	**335**	**190**

Table 89 Fetal maceration in cases of non SGA and SGA. Excludes 8 cases with no description of maceration. Significantly more macerated fetuses in the SGA population (z=2.571 p=0.0102)

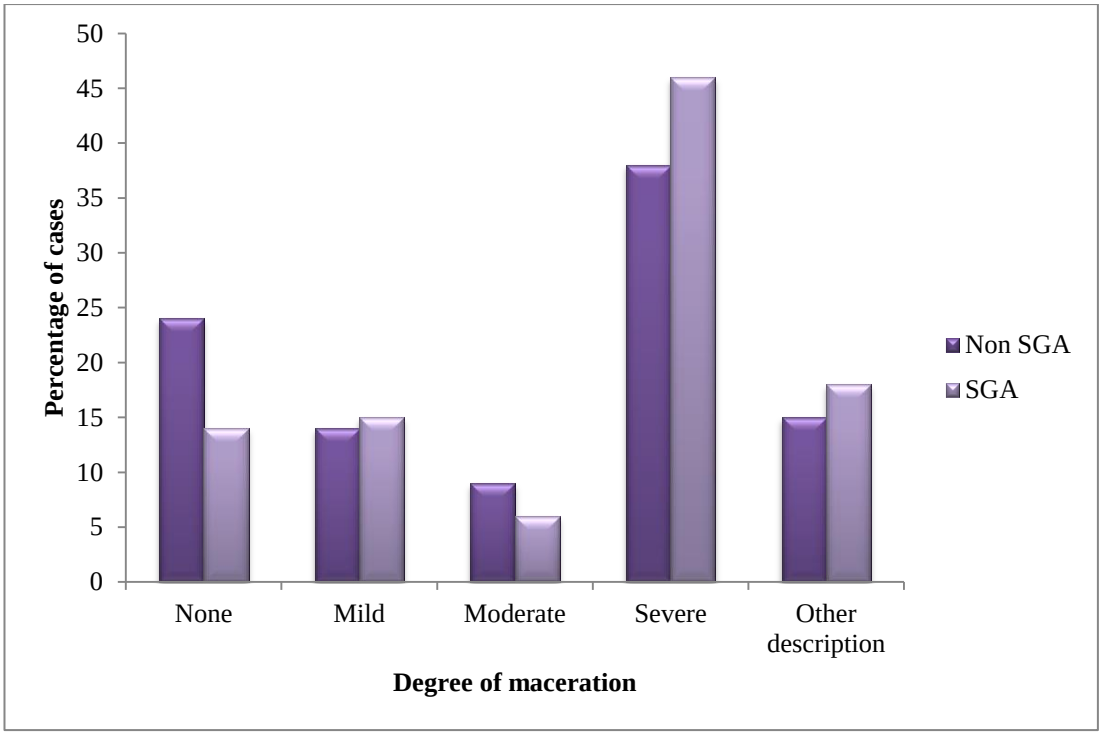


Figure 49 *Fetal maceration in cases of non SGA and SGA. Significantly greater proportion of macerated fetuses in the SGA population (z=2.578 p=0.0099)*

Excluding cases of Ascending Infection

Fetal Maceration	Non SGA	SGA	**Total:**
None	56 (19%)	21 (12%)	**77 (16%)**
Mild	39 (13%)	24 (13%)	**63 (13%)**
Moderate	29 (10%)	12 (7%)	**41 (9%)**
Severe	123 (41%)	88 (49%)	**211 (44%)**
Other description	50 (17%)	33 (19%)	**83 (17%)**
Total:	**297**	**178**	**475**
Not given	6	2	

Table 90 Fetal maceration in cases of non SGA and SGA. Excluding all cases of ascending infection.

When cases of ascending infection were excluded, there continued to be a significantly greater proportion of macerated cases in the SGA population than in the non-SGA population (z=2.020 p=0.0434), indicating that maceration itself is

associated with reduced body weight for gestational age rather than SGA fetuses dying in utero and hence being macerated (See Chapter 6 on organ weights for further details).

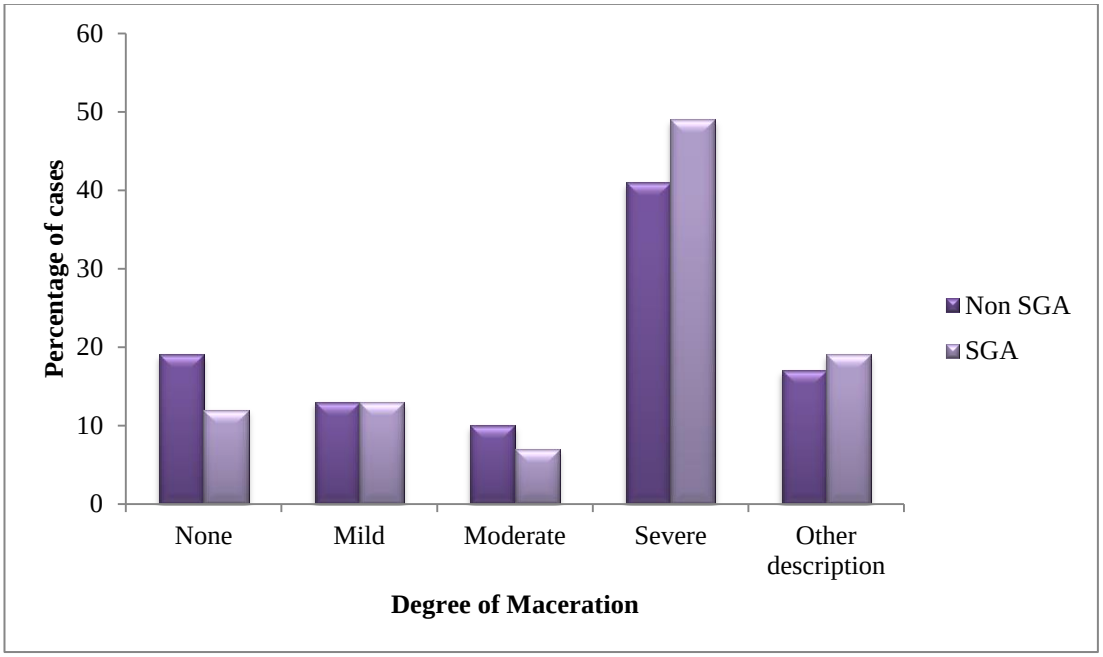


Figure 50 *Fetal maceration in cases of non SGA and SGA. Excluding all cases of ascending infection. There was a significantly greater proportion of macerated cases in the SGA population than in the non-SGA population (z=2.020 p=0.0434).*

5.3.9 Effect of intrauterine and postmortem intervals on fetal weight and implications for SGA diagnosis at autopsy

Intrauterine interval (IUI) was calculated as a minimum value according to the antenatal notes i.e. the fetus had been dead in utero for at least X number of days according to either the last fetal ultrasound scan results or fetal movements felt by the mother. Fresh intrapartum deaths were recorded as having 0 days intrauterine interval.

431 of 1,064 cases had a recorded birthweight, body weight and well documented intrauterine interval, of which 308 were >23 weeks of gestation. Seven cases were excluded for not having a status of maceration recorded. 103 cases were SGA. There

was a relationship, between increasing intrauterine interval and more severe maceration. (*Figure 51)* (z=15.45, p<0.0001). However, maceration severity was not closely related to IUI; whilst all cases with prolonged (>4 days) documented IUI demonstrated moderate or severe maceration, so did some cases with an IUI of only two days. Presence of significant maceration appears to be a reliable indicator of intrauterine death prior to delivery, with retention of at least 24-48 hours, but cannot be used to determine the exact period since death and delivery.

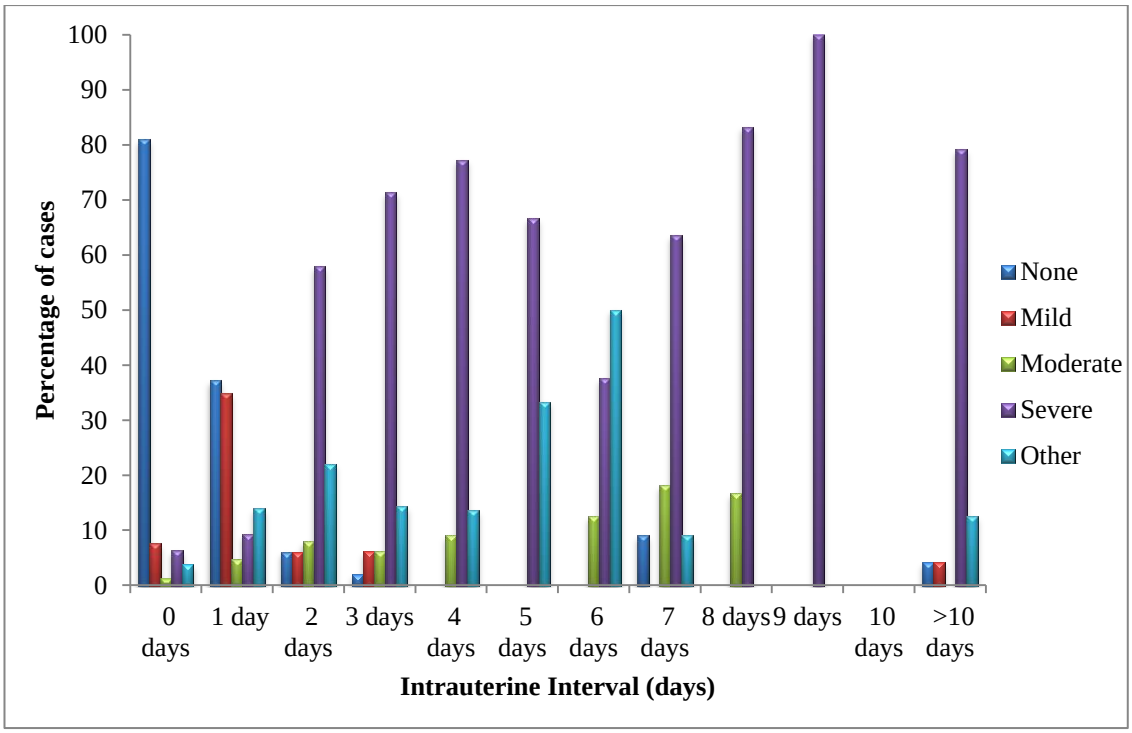


Figure 51 *Percentage of cases with different degrees of maceration according to intrauterine interval. With increasing IUI, maceration becomes more severe (z=15.45, p<0.0001).*

In addition, there was a significant relationship between intrauterine interval and delta birth weight (*Figure 52)* (p<0.0001). As the intrauterine interval increased, the delta birth weight value decreased (became more negative) indicating that with intrauterine retention the fetus loses weight in utero. A. prolonged intrauterine interval therefore affects fetal birthweight and hence the proportion of SGA.

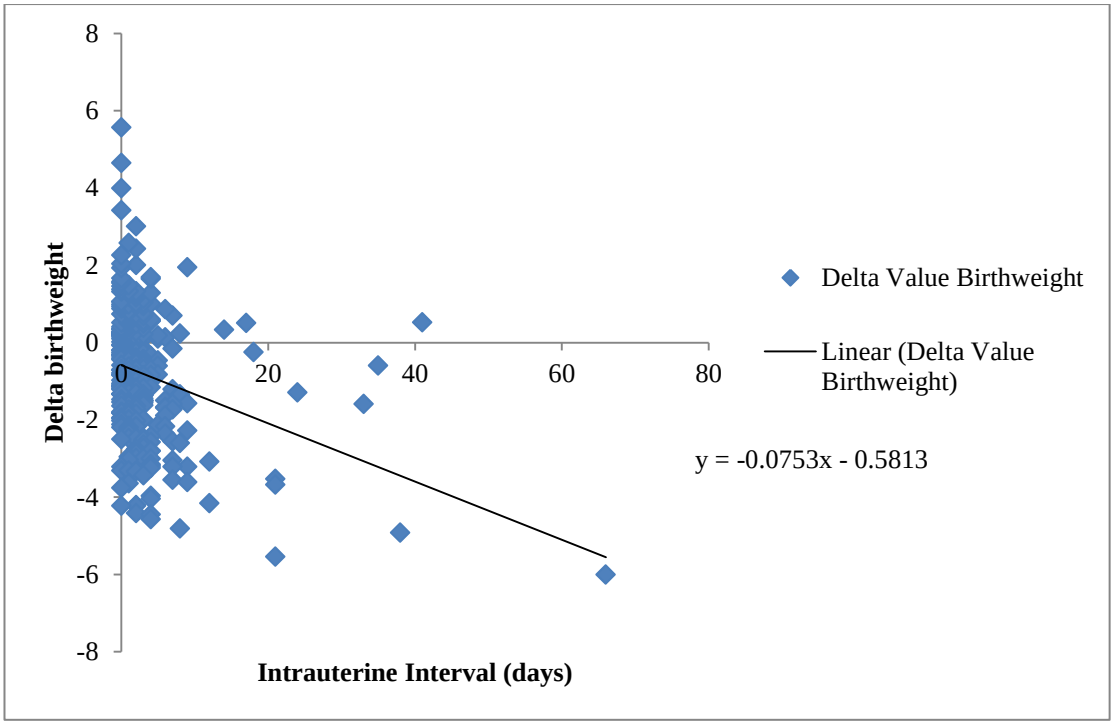


Figure 52 *The significant linear relationship between intrauterine interval and delta birthweight (p< 0.0001)*

The proportion of SGA cases in stillbirths > 23 weeks was 21% in those with 0 days IUI, 30% in those with 1-2 days IUI and 44% in those > 2 days IUI. There were significantly more fetuses with an IUI of >2 days in the SGA group (z=3.324, p = 0.0009) compared to the non-SGA population (*Table* 91).

Intrauterine Interval	Non SGA	SGA	**Total**
0 days	60 (29%)	16 (16%)	**76 (25%)**
1- 2 days	80 (39%)	35 (34%)	**115 (37%)**
> 2 days	65 (32%)	52 (51%)	**117 (38%)**
Total:	**205**	**103**	**308**

Table 91 Intrauterine interval in non SGA and SGA cases. Significantly more fetuses with a prolonged IUI (> 2 days) in the SGA group (z=3.324 p=0.0009)

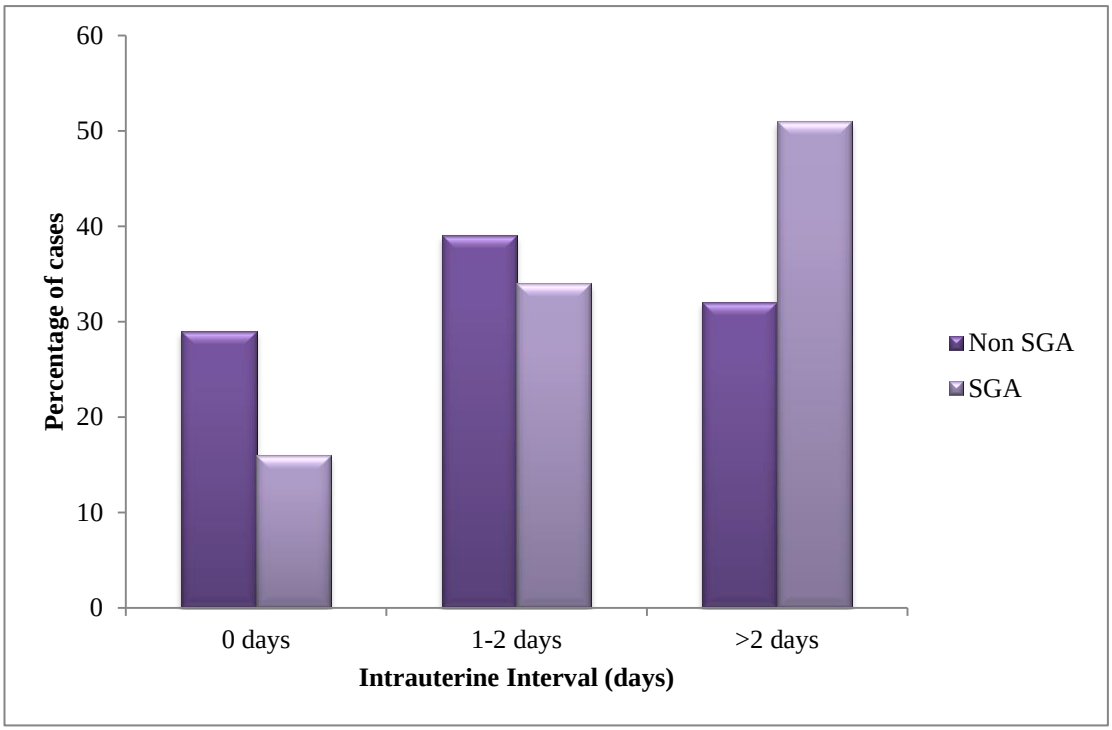


Figure 53 *Intrauterine interval in non SGA and SGA cases. Significantly more fetuses with a prolonged IUI (> 2 days) in the SGA group (z=3.324 p=0.0009).*

5.3.10 Postmortem interval and birth weight/ body weight differences

615 cases had both reliably recorded birthweight and body-weight at autopsy to allow for the calculation of fetal weight change over the time period between birth and autopsy (.i.e. the postmortem interval (PMI) (*Figure 54*).

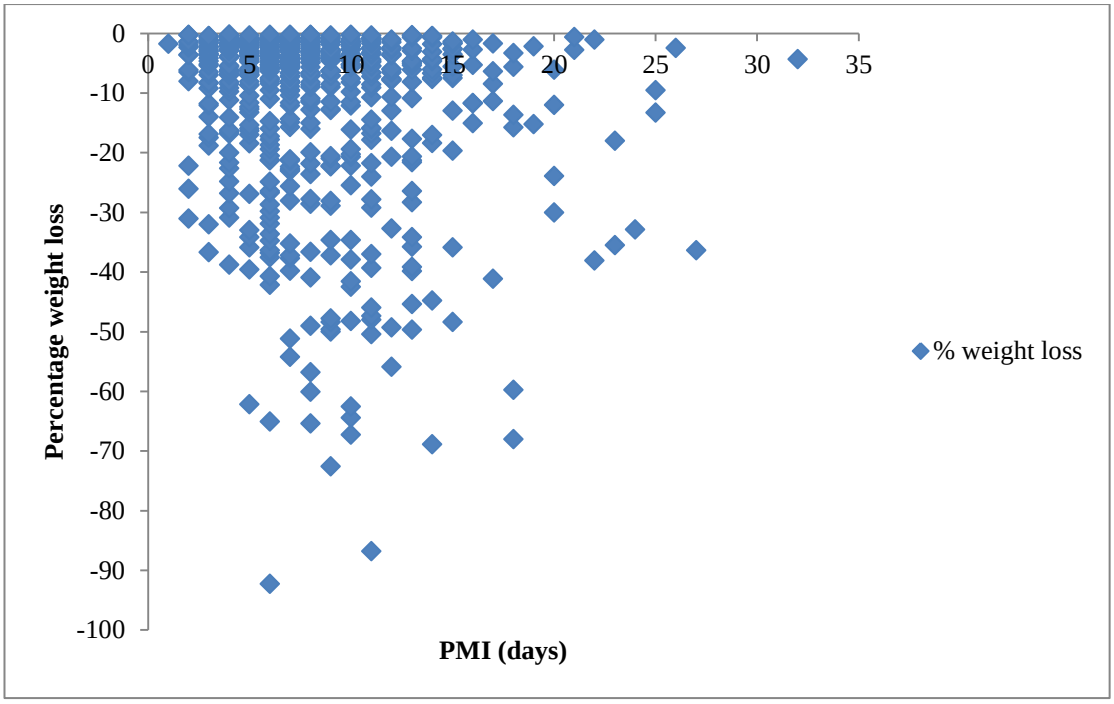


Figure 54 *Postmortem interval and recorded percentage fetal weight loss in all cases (excludes 369 cases with no birth weight, 57 typed errors and 11 cases which lost apparently> 92% of their birth weight and 1 case with a PMI of 79 days))*

There was an average 12% loss of fetal birthweight whilst the fetus was refrigerated pending autopsy and an average PMI of 8 days. As PMI increased the average percentage fetal weight loss significantly increased (p = 0.0001) (*Table 92 and Figure 55*)

PM Interval	Average % weight loss
1 Day	-1.76
2 Days	-8.21
3 days	-7.27
4 days	-8.32
5 days	-8.53
6 days	-12.78
7 days	-9.11
8 days	-11.36
9 days	-13.99
10 days	-15.19
>10 days	-15.19

Table 92 Average percentage weight loss(all cases). As PMI increases percentage weight loss increases (p = 0.0001)

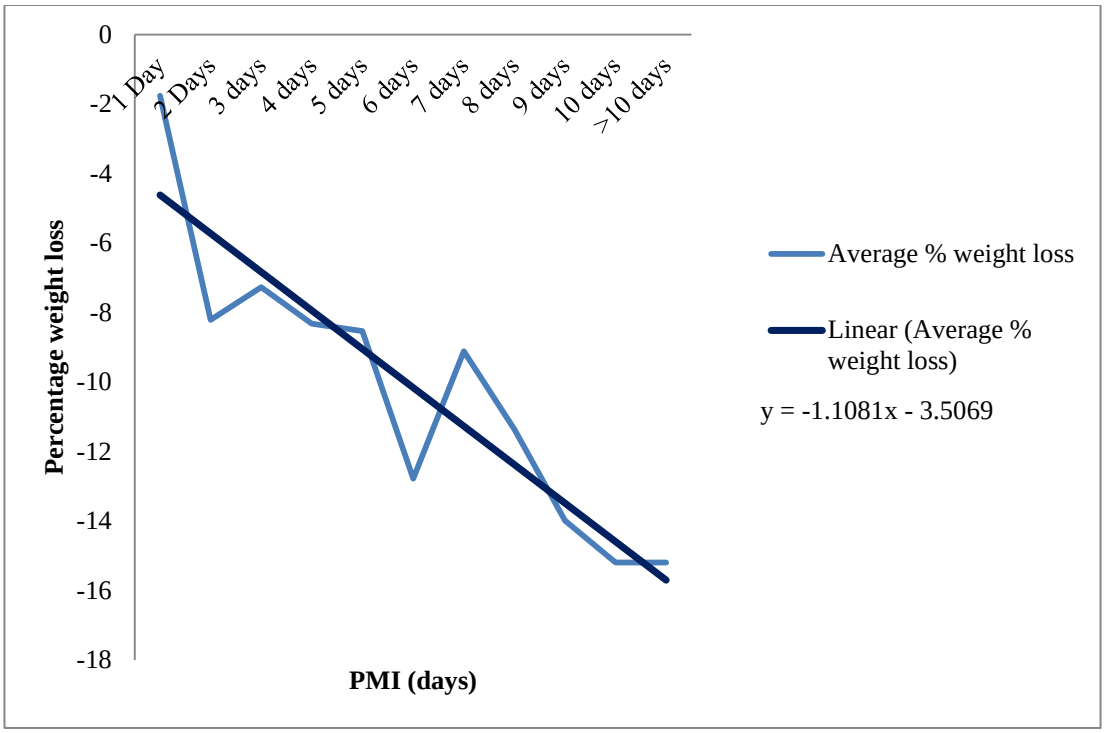


Figure 55 *Average percentage weight loss in all cases. AS PM interval increases percentage weight loss increases (p= 0.0001)*

Furthermore, calculating delta body weight (using the same method as used to calculate delta birthweight), 255 cases were classified as SGA; a 12% rise compared to using delta birth weight for the calculation.

5.4 Discussion

The findings of this chapter have demonstrated that, based on birthweight against standard fetal size charts, 36% of stillbirths >23 weeks of gestation initially appear to be SGA (38% male and 34% female); significantly greater than the expected 10%. Similar percentages were reported in a smaller UK study in which 44% were SGA, after a detailed review of the notes and the use of customized growth charts. Another study, using the ReCoDe classification, found 43% of stillbirths to be SGA (5, 207). A French study reported 49% of cases as SGA (208). However, within the same study, when the hierarchy of SGA was reduced such that low birthweight for gestational age was retained only in the absence of other conditions, the proportion of stillbirths assigned to the FGR category decreased from 38.2 to 14% (208).

The majority of SGA fetuses in the present study (48% male and 46% of females) had no specific identifiable cause of death at autopsy and were hence classified as Unexplained. However, of the total number of unexplained deaths in the study, only around 27% were SGA.

The most common specific findings in cases with known IUGR or SGA were abnormalities of the placenta, mainly changes suggestive of uteroplacental malperfusion with other specific causes including abruption and infection. These figures are similar to the French stillbirth study outlined above, in which 25.5% of the SGA cases had a placental cause of death (208). The literature review reveals very few studies which report, in detail, the causes of death within the subcategory of stillborn fetuses born SGA. This may be because one of the most frequently used classification systems (ReCoDe) uses a separate category for FGR (in reality SGA) and thus the mechanism of death in such cases is assumed to be growth restriction and not reviewed further (5). The present study highlights that although the fetus

may be SGA, or even FGR, this alone may not be the cause of death. Indeed, 60% of the cases identified antenatally with FGR had a specific or alternative cause of death at autopsy including, placental abruption, ascending infection, congenital abnormalities, other abnormalities within the placenta or feto-maternal haemorrhage.

5.4.1 Estimation of fetal size is affected by changes occurring after death

Maceration, the process of fetal decomposition after intrauterine death, is rarely investigated or analysed in current practise (167). Within this study, 89% of SGA fetuses showed some degree of maceration: a significantly higher proportion than non SGA fetuses. In addition, as the intrauterine interval increased, the more macerated the fetus appeared at autopsy. Furthermore, as the intrauterine interval increased, fetal delta birthweight decreased, indicating that fetuses progressively lose weight following death in utero. Therefore if birthweight is considered without adjustment for effects of intrauterine interval and fetal maceration, the proportion of SGA cases will inevitably be erroneously overestimated. Finally, fetal weight also further decreased during the postmortem interval with an average 12% loss of birthweight between delivery and autopsy. If delta body weight at autopsy is used instead of delta birth weight to assess for SGA, there will be a further > 10% rise in the number of fetuses diagnosed with SGA, all erroneously, highlighting that pathologists need to carefully consider which weight they use to assess for SGA and account for post-death changes. (See also chapter regarding organ weights and weight ratios for diagnosis of IUGR and effects of postmortem changes).

These data suggest that, overall, around 30-40% of stillbirths initially appear to be SGA, but many of these are erroneously classified due to the effects of postmortem and post-delivery changes. Since around half of all SGA fetuses probably represent physiological small size, and the current data indicate that around 20% of apparent

SGA fetuses are erroneously classified as such due to postdeath changes, it is estimated that around 10-20% of clinically unexplained stillbirths may be related to pathological growth restriction (209). This has implications for the maximum likely effects of major National programmes to increase detection of growth restriction to reduce stillbirth rates.

In conclusion, this chapter has demonstrated that:

- Based purely on unadjusted birthweight centiles, around 35% of stillbirths are SGA.
- There is a significant association between increasing maceration and increasing rate of SGA.
- The longer the intrauterine interval, the greater the degree of fetal maceration and the more delta birthweight decreases.
- Fetuses lose on average 12% of their birthweight in the interval between delivery and autopsy.
- Without adjusting for such factors, using birthweight or bodyweight alone will erroneously significantly overestimate the role of SGA as an underlying factor in stillbirth causation.

To further assess the relationship between stillbirth and FGR/ SGA, further research is required into the biological and pathological mechanisms of death in FGR/SGA, accounting for the relationship between the intrauterine interval, postmortem interval, maceration and fetal birthweight.

6. Organ Weights

6.0 Background

6.1 Chapter Aims

6.2 Methods

- 6.2.1 Calculating Delta Organ weights

6.3 Numbers of organs analysed

6.4 Results

- 6.4.1 Significant results
- 6.4.2 Ratio calculations
- 6.4.3 Significant results
- 6.4.4 Brain:Liver weight ratio
- 6.4.5 Body:Thymus weight ratio

6.5 Discussion

6.0 Background

The Royal College of Pathologists recommends the weighing of all major organs at autopsy in cases of stillbirth/ miscarriage; these guidelines based primarily on expert opinion rather than published data (159). Only one published study reviews the effects of a maternal factor - maternal diabetes mellitus- on fetal organ weights; a surprising find when it is known that other maternal factors such as body mass index and ethnicity are associated with stillbirth (52, 94, 95, 210).

There are also no published studies which review the effects on organ weights of different causes of death in stillbirth, (other than brain:liver weight ratio in IUGR see below), and hence the potential usefulness of organ weight metrics for determining cause or mode of death. Furthermore, the postmortem brain:liver weight ratio is commonly used as a marker of nutrition and growth in fetuses but this is based on limited data. Finally, few published studies have examined the effects of intrauterine retention, fetal maceration and postmortem interval on organ weights at autopsy and therefore - possible effects on brain:liver weight ratio (169, 170, 211). From results demonstrated previously within this thesis (Chapter 5) it has been established that without adjustment for effects of intrauterine interval and fetal maceration, the proportion of Small for Gestational Age (SGA) cases will be erroneously overestimated, based on either both fetal birthweight and especially bodyweight recorded at autopsy. If maceration changes also affect organ weights, especially if this effect is variable by organ, organ weights and ratios such as the brain: liver weight ratio, such weights and ratios will not be an accurate way to diagnose fetal malnourishment or IUGR in this setting.

6.1 Chapter Aims

The aims of this Chapter are therefore to examine whether there are any significant differences or relationships between gestational age and sex adjusted fetal organ weights (see methods) and:

1. Maternal body mass Index
2. Maternal Hypertension
3. Maternal Diabetes Mellitus
4. Small for gestation age fetuses
5. The cause of death allocated at autopsy
6. Intratuterine retention and fetal maceration

In cases where a significant relationship exists, body:organ weight ratios will be calculated to assess whether organs are disproportionately lighter compared to body weight or changes in organ weights are simply proportional to overall changes in fetal body weight. Brain:liver weight ratio will also be examined in detail including its relationship to fetal maceration, intrauterine growth restriction/ SGA and cause of fetal death.

6.2 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths and early and late miscarriages from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Data was analysed through queries and statistical tests run using Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Statistical tests can be viewed in detail in appendix 3.

6.2.1Calculating Delta Organ weights

1. Female and male fetal organ weights were plotted separately against the fetal gestation for each case
2. Polynomial regression calculations assessed the trend and were used to calculate organ weights on the 50th centile
3. Using Stats Direct the polynomial regression equation was interpolated X to Y to determine the 2.5th centile for each gestation
4. The standard deviation in grams for each gestation was calculated by:

$$\frac{(50^{th}\text{ centile value} - 2.5^{th}\text{ centile value})}{1.96}$$

5. The delta value for each organ was then calculated by:

$$\frac{\text{Observed organ weight (g)} - \text{Expected organ weight (g) } (50^{th}\text{ centile})}{\text{Standard deviation (g)}}$$

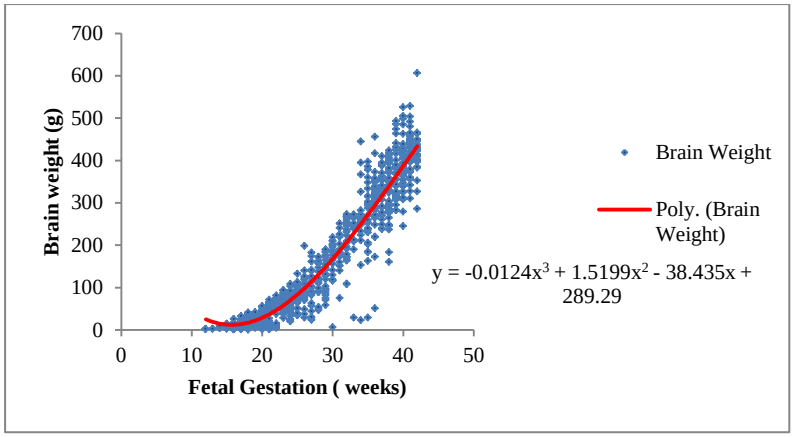
Calculating a delta organ weights allows for the comparison of organ weights between different clinical groups, without the confounding factors of gestational age or fetal sex.

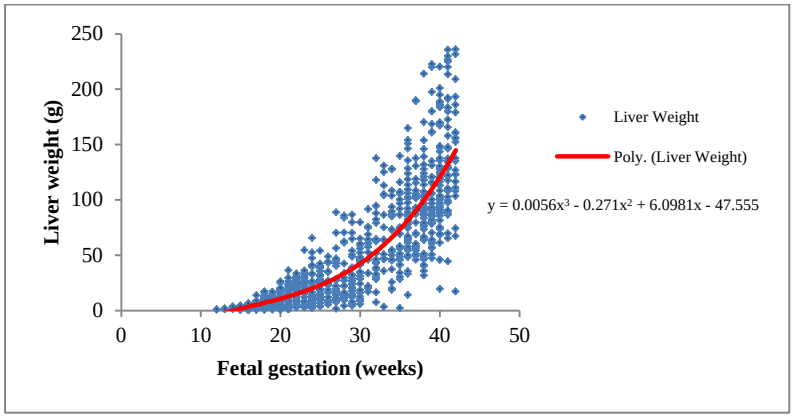
6.3 Organs analysed

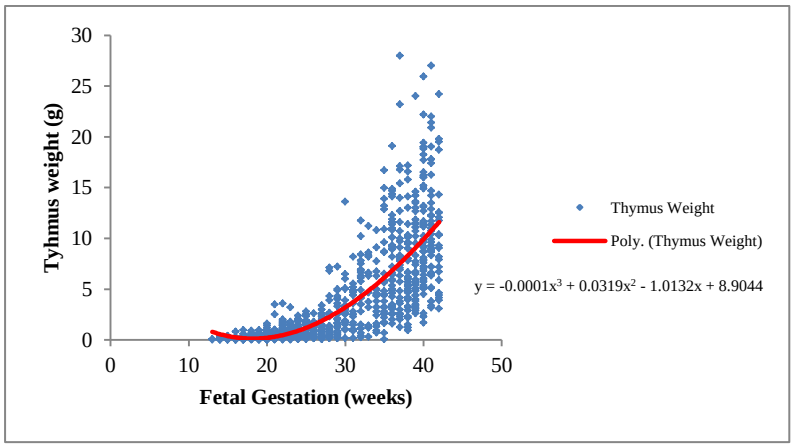
Within the study:

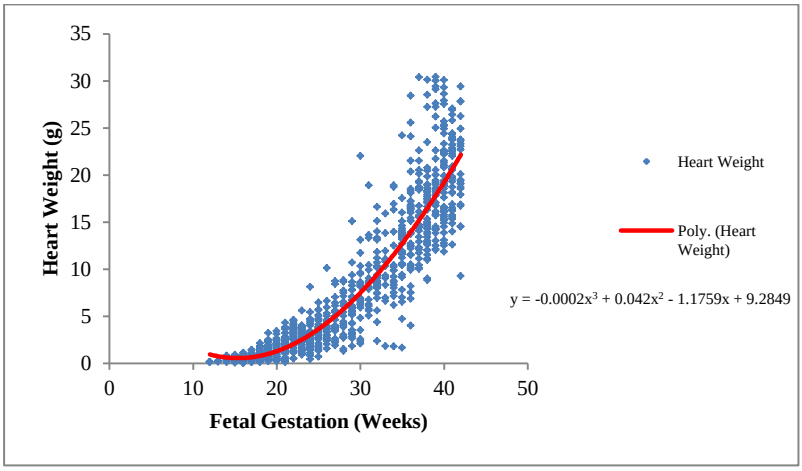
- 911 brains weighed and 52 excluded from analysis (33 no gestation and 19 typed errors/ outliers)
- 957 livers weighed and 41 excluded from analysis (34 no gestation and 7 typed errors/outliers)
- 930 thymus' weighed and 36 excluded from analysis (33 no gestation and 3 typed errors/outliers)
- 967 hearts weighed and 36 excluded from analysis (34 no gestation and 2 typed errors/outliers)
- 313 combined lungs weighed and 38 excluded from analysis (37 no gestation and 1 typed error/outlier)
- 406 combined kidneys weighed and 43 excluded from analysis (37 no gestation and 6 typed errors/outliers)
- 913 spleens weighed and 42 cases were excluded from analysis (36 no gestation and 6 typed errors/outliers)
- 952 combined adrenals weighed and 38 excluded from analysis (Excludes: 33 no gestation and 5 typed errors/outliers)
- 745 pancreas' weighed and 39 excluded from analysis (36 no gestation and 3 typed errors/outliers)
- 154 thyroids weighed and 34 excluded from analysis (33 no gestation and 1 typed error/outlier)

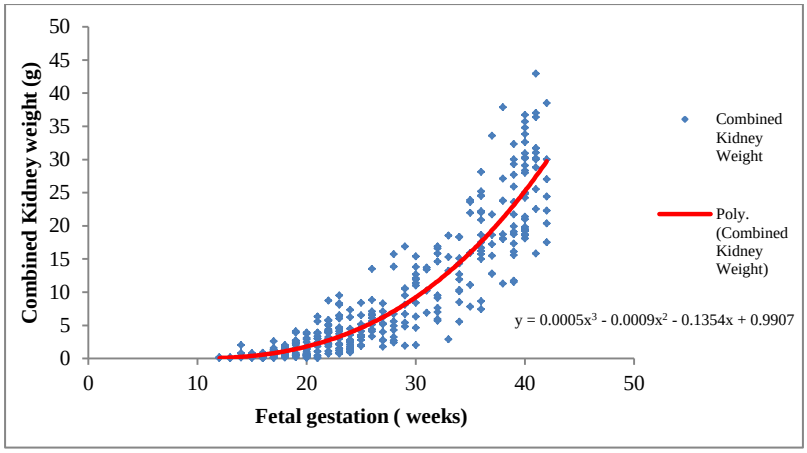
(NB: For the purposes of this study, typed error/ outliers were those in which the recorded organ weight was non-physiological, usually an order of magnitude larger or smaller than expected suggesting a transcription error or a missing or additional decimal place)

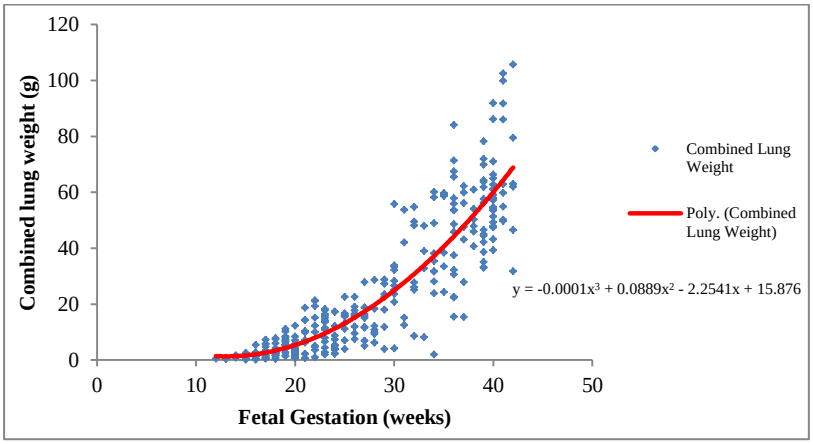

Brain weight (g)
700
600
500
400
300
200
100
0
0
10
20
30
40
50
Fetal Gestation (weeks)
Brain Weight
Poly. (Brain Weight)
y = -0.0124x³ + 1.5199x² - 38.435x + 289.29

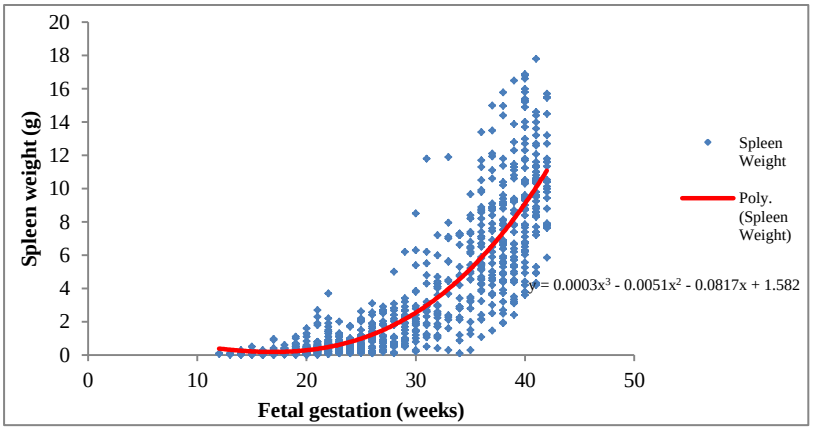

Liver weight (g)
250
200
150
100
50
0
0
10
20
30
40
50
Fetal gestation (weeks)
Liver Weight
Poly. (Liver Weight)
y = 0.0056x³ - 0.271x² + 6.0981x - 47.555

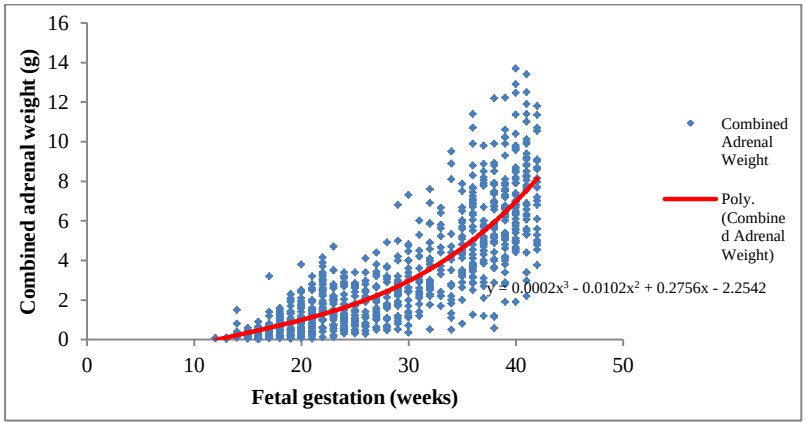

Tyhmus weight (g)
30
25
20
15
10
5
0
0
10
20
30
40
50
Fetal Gestation (weeks)
Thymus Weight
Poly. (Thymus Weight)
y = -0.0001x³ + 0.0319x² - 1.0132x + 8.9044


Heart Weight (g)
35
30
25
20
15
10
5
0
0
10
20
30
40
50
Fetal Gestation (Weeks)
Heart Weight
Poly. (Heart Weight)
y = -0.0002x³ + 0.042x² - 1.1759x + 9.2849


Combined Kidney weight (g)
Fetal gestation (weeks)
Combined Kidney Weight
Poly. (Combined Kidney Weight)
y = 0.0005x³ - 0.0009x² - 0.1354x + 0.9907


Combined lung weight (g)
Fetal Gestation (weeks)
Combined Lung Weight
Poly. (Combined Lung Weight)
y = -0.0001x³ + 0.0889x² - 2.2541x + 15.876


Spleen weight (g)
Fetal gestation (weeks)
Spleen Weight
Poly. (Spleen Weight)
y = 0.0003x³ - 0.0051x² - 0.0817x + 1.582


Combined adrenal weight (g)
Fetal gestation (weeks)
Combined Adrenal Weight
Poly. (Combined Adrenal Weight)
y = 0.0002x³ - 0.0102x² + 0.2756x - 2.2542

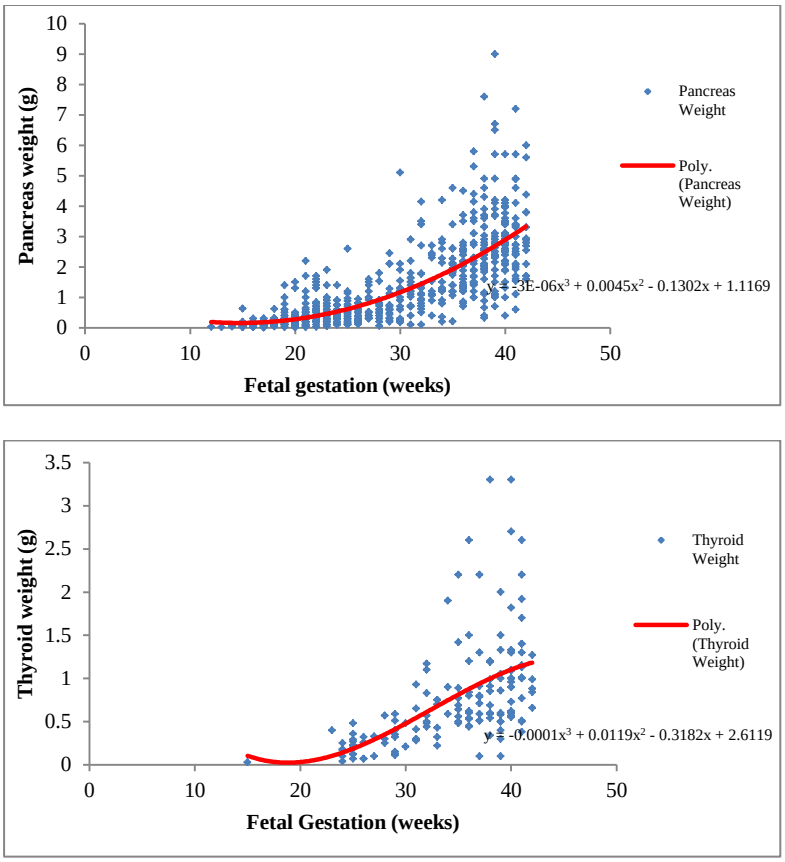


Figure 56, Figure 57, Figure 58, Figure 59, Figure 60, Figure 61, Figure 62, Figure 63, Figure 64, Figure 65 *Polynomial regression of fetal gestation and organ weights for males and females (Brain, Liver, Thymus, Heart, Combined kidneys, Combined Lungs, Spleen, Pancreas and Thyroid).*

6.4 Results

Each organ was statistically analysed over six different categories and assessed separately for male and female fetuses:

1. *Body Mass Index:* The comparison of delta organ weights in mothers with Normal (N) BMI versus Obese and Overweight (OW) BMI and underweight (UW) BMI versus Obese

2. *Hypertension:* The comparison of organ delta weights for those mothers with versus those mothers without hypertension.

3. *Diabetes Mellitus:* The comparison of delta organ weights in mothers with versus those without any form of Diabetes Mellitus.

4. *Small for gestational age (SGA):* The comparison of organ delta weights in those fetuses with delta birthweights less than the 10^{th} centile (<-1.375) versus those with birthweight delta values greater than the 10^{th} centile (> -1.375).

5. *Cause of death:* The comparison of organ delta weights in cases of ascending infection versus unexplained cases and in placental causes of death versus unexplained causes of death (since these categories were the most frequent causes of death in Chapter 4).

6. *Maceration:* The comparison of organ delta weights in those cases with maceration versus those with no maceration.

6.4.1 Significant results

- *Diabetes Mellitus:* Fetuses of diabetic mothers had heavier adrenal glands than non-diabetic mothers (males p=0.0279, females p=0.038)

- *Small for gestational age (SGA):* Most organs (brain, liver, heart, thymus, lungs, kidneys and thyroid) were significantly lighter in SGA fetuses than non SGA fetuses (Males and females p<0.0001, p<0.0001, p<0.0001, p<0.0001, p<0.0001, p<0.0001, male thyroid p= 0.0299, female thyroid p=0.0027 respectively).

- *Cause of death:*

    - The liver was lighter in cases of placental causes of death than in unexplained death ($p<0.0001$) and heavier in cases of ascending infection than unexplained death (males: $p= 0.0032$, females: $p<0.0001$).
    - The adrenal glands were heavier in placental causes of death than in unexplained causes of death and heavier in cases of ascending infection than in unexplained death (males $p=0.0003$ and $p= 0.0214$ and females $p<0.0001$ respectively).
    - The thymus gland was lighter in placental causes of death compared to unexplained deaths (males $p=0.0001$ and females $p=0.0069$).

- M*aceration:* Most organs (brain, liver, thymus, heart, lungs, adrenals, and thyroid) were lighter in macerated fetuses compared to non-macerated (males $p=0.0008$, $p<0.0001$, $p<0.0001$, $p=0.0003$, $p=0.0081$, $p=0.0066$, $p= 0.0065$ and females $p< 0.0001$ for all except thyroid where $p=0.0069$).

Some changes were noted only for male or female fetuses and are therefore of uncertain significance.

- *Body mass index*: In female fetuses only overweight mothers delivered fetuses with heavier hearts than mothers with a normal BMI ($p=0.0427$)

- *Hypertension:* In female fetuses only, mothers with hypertension delivered fetuses with lighter spleens compared to non-hypertensive mothers ($p=0.0091$)

- *Cause of death:* In female fetuses only the brain, heart lungs, kidneys and spleen were heavier in cases if ascending infection than in unexplained death ($p=0.0249$, $p<0.0001$, $p<0.0001$, $p<0.0001$ and $p=0.0023$ respectively) and the spleen was lighter in placental causes of death than in unexplained death ($p=0.0023$); but the pancreas was heavier in cases of placental causes of death than in unexplained death ($p=0.0002$). In males only the brain, heart lungs and kidneys were lighter in placental causes of death than in unexplained death ($p<0.0001$, $p=0.0005$, $p=0.0006$, $p<0.0001$ respectively)

- *Maceration:* In female fetuses the kidney, spleen and pancreas were also significantly lighter in macerated fetuses compared to non-macerated ($p< 0.0001$ for kidney and spleen and $p=0.0001$ for pancreas).

All other comparisons showed no significant differences.

6.4.2 Ratio calculations

Body:organ weight ratios were calculated - for those organs with significant results detailed above- in relation to maceration, cause of death and SGA categories, for male and female fetuses. The aim of assessing these ratios was to establish;

1. In cases of maceration, were organ weights lighter because the organs themselves were selectively degenerate (disproportionally lighter) or were the organs proportionally lighter simply because macerated fetuses had lower overall body weight.

2. In different causes of death, were the organs lighter because of effects of the cause of death on individual organs or was the whole fetus symmetrically smaller as an effect of the cause of death

3. In cases of SGA, were organs weights lighter because the organs themselves were pathologically small (disproportionally lighter) or were the organs proportionally lighter simply because SGA fetuses had lower body weight.

Polynomial and linear regression analysis found that none of these ratios were significantly associated with gestational age over the range assessed and thus original (observed weight in grams) ratios were used.

6.4.3 Significant Results

SGA

1. Body:organ weight ratios for the thymus, liver and spleen were significantly greater in cases of SGA compared to non SGA (males: $p<0.0001$, $p<0.0001$, $p=0.0002$, females: $p<0.0001$, $p<0.0001$, $p=0.0063$)
    a. **Finding:** SGA fetuses have disproportionally lighter thymuses, livers and spleens
    b. **Implication:** The thymus, liver and spleen preferentially lose weight more than the body in cases of SGA

2. In males and females Brain:Thymus weight ratio was significantly greater in SGA fetuses compared to non SGA fetuses ($p<0.0001$ in both sexes)
    a. **Finding:** SGA fetuses have disproportionally lighter thymuses compared to brains.
    b. **Implication:** The thymus preferentially loses weight more than the brain in cases of SGA

All other male and female organs had non-significant p values comparing organ weight ratios in SGA and Non SGA fetuses or changes only present in one sex and were thus excluded.

Cause of death

1. The Body:Brain and Body:Liver weight ratios are significantly greater in cases of unexplained causes of death compared to cases of ascending infection (Males: $p=0.0017$ and $p<0.0001$ respectively females: $p= 0.0008$ and $p<0.0001$ respectively)
    a. Fetuses with unexplained death have disproportionally lighter brains and livers, or cases of ascending infection have disproportionally heavier brains and livers.

However, to address this, when comparing only non-macerated unexplained deaths, with cases of non-macerated ascending infection, these organs weight ratios are no longer significantly different. The lungs, however are still significantly heavier in the cases of ascending infection likely due to pneumonia and infiltration of the lungs of neutrophils and congestion. These findings suggest that in general it is maceration that is causing the difference in results, not the mechanism of death.

2. The Body:Liver and Body:Thymus weight ratios are significantly greater with placental causes of death compared to unexplained death (Males: $p<0.0001$ and $p=0.0008$ respectively Females: $p<0.0001$ and $p=0.0166$ respectively)
    a. Fetuses with a placental mechanism of death have disproportionally lighter livers and lighter thymuses.

Maceration

Male and female Body:Organ weight ratios for most organs (liver, thymus, lung, pancreas, adrenal gland, kidney, heart), were significantly higher in macerated fetuses compared to non-macerated fetuses (males: $p<0.0001$, $p=0.0045$, $p=0.0086$, $p=0.0033$, $p<0.0001$, $p=0.0032$, , $p=0.0096$ respectively females: $p<0.0001$, $p=0.0049$, $p<0.0001$, $p=0.0187$, $p<0.0001$, $p<0.000$, $p<0.0001$ respectively). Note that overall, the brain weight ratio was not significantly affected.

a. **Finding:** Macerated fetuses have disproportionally lighter organs
b. **Implication:** Organs preferentially lose weight more than the musculoskeletal elements of the body with maceration

(Males body:thyroid and female body:brain weight ratios were also significantly higher in macerated fetuses compared to non-macerated fetuses ($p= 0.0014$ and $p=0.0017$ respectively) but are unlikely to represent true findings as they were not present in both sexes).

The female spleen and thyroid gland showed no significant difference between ratios in macerated and non-macerated fetuses.

Figure 66 In the non-macerated fetus organs are of usual proportions.

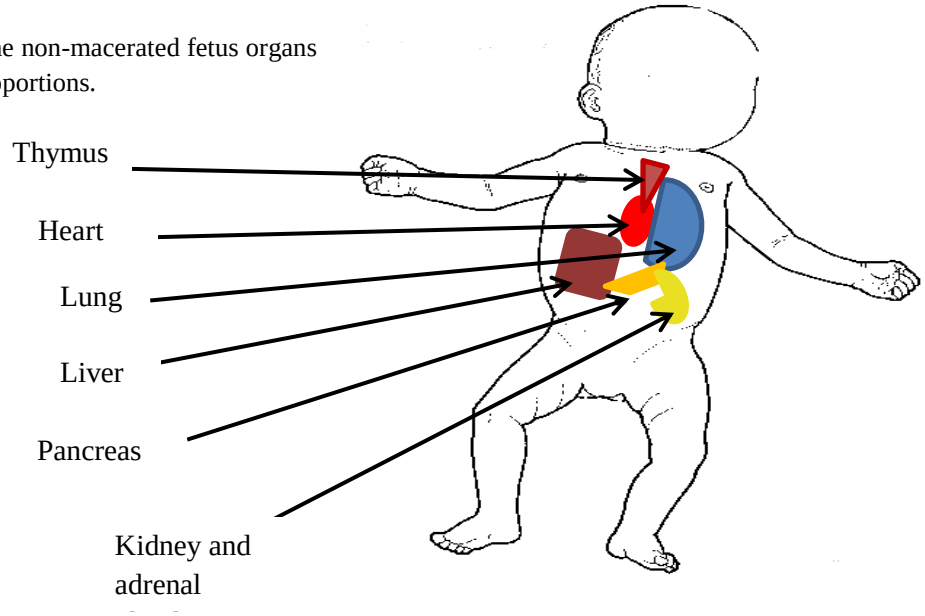
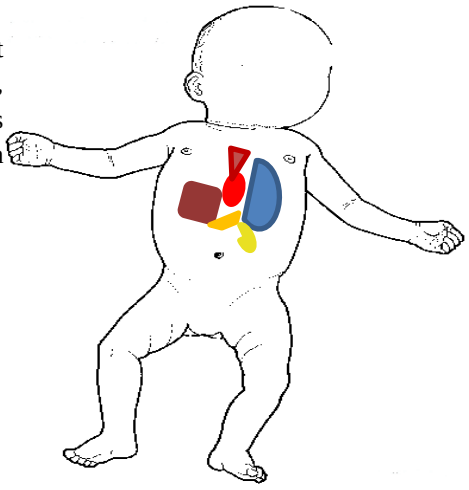


Figure 67 With maceration the fetus is lighter but internal thoracoabdominal organs such as the liver, thymus, lungs, pancreas, adrenal glands, kidneys and heart are all disproportionally reduced in weight in relation to the body.



(212)

Further points

There was no significant difference in body:liver, body:heart or body:adrenal weight ratios according to maternal body mass index.

6.4.4 Brain:Liver weight ratio

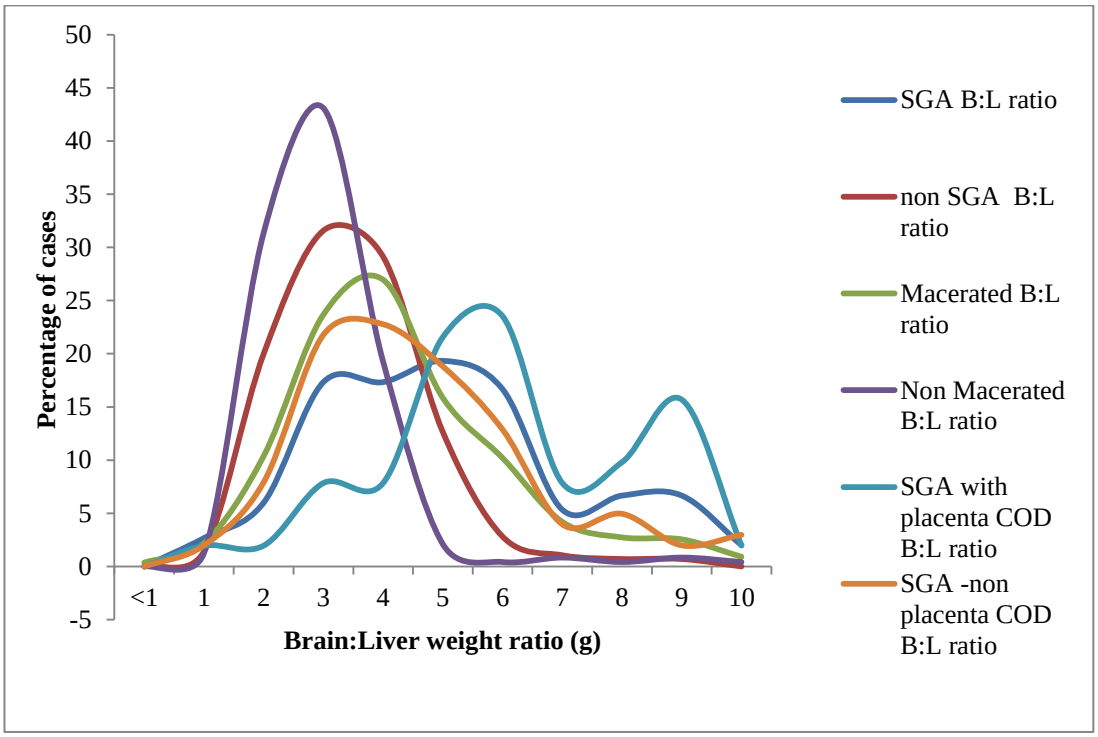


Figure 68 *Proportion of cases with specific Brain:Liver weight ratios (males and females) in cases of SGA; non SGA; macerated and non-macerated; SGA cases with a placental cause of death and SGA without a placental cause of death. (Excludes 27 cases with a brain:liver weight ratio > 10).*

There were significant associations between Brain:Liver weight ratio (for males and females) and fetal maceration and specific causes of death specifically:

1. Brain:Liver weight ratio distribution is significantly greater in macerated fetuses compared to non-macerated fetuses ($p<0.0001$ in both sexes). Median 3.94 (range 0.09-9.97) versus median 2.78 (range 1.17-9.95), median difference 1.08 (95% CI 0.90 to 1.27).
    c. **Finding:** Macerated fetuses have disproportionally lighter livers in relation to their brains than non-macerated fetuses
    d. **Implication:** The liver preferentially loses weight more than the brain in cases of maceration and the Brain:Liver weight ratio is therefore artefactually increased due to the secondary effects of maceration rather than IUGR.

Brain:Liver weight ratio remains significantly greater for macerated unexplained deaths compared to non-macerated unexplained deaths ($p<0.0001$). Median 3.84 (range 0.09-9.88) versus median 3.05 (range 1.47-9.95), median difference 0.77 (95% CI 0.52 – 1.04). In macerated stillbirths the Brain:Liver weight ratio should therefore be adjusted by 0.8 to take into account the effects of maceration.

2. Brain:Liver weight ratio is significantly greater in non-macerated unexplained deaths compared to non-macerated ascending infection deaths ($p= 0.003$). Median 3.05 (range 1.47-9.95) versus median 2.65 (range 1.32 - 4.38), median difference 0.40 (95% CI 0.19 -0.62).
    a. **Finding:** Unexplained deaths have lighter livers than cases of ascending infection.
    b. Implication: Unexplained deaths probably contain a proportion of unidentified IUGR cases with smaller livers than cases of ascending infection or ascending infection is associated with brain swelling. (NB: Delta Brain weight and delta liver weight are both significantly lighter in unexplained deaths compared to cases of ascending infection suggesting both factors contribute to the ratio difference)

3. Brain:Liver weight ratio is significantly greater in SGA fetuses compared to non SGA fetuses ($p<0.0001$ in both sexes). Median 4.84 (range 0.51-9.88) versus median 3.42 (range 1.32- 8.92), median difference 1.38 (95% CI 1.03-1.72).
    a. **Finding:** SGA fetuses have a disproportionally lighter liver in relation to their brain weight.

b. **Implication**: The liver preferentially gains weight less than the brain in cases of SGA

4. If cases of SGA are divided into "true" IUGR i.e. those with a placental cause of death such as abruption, known IUGR, Placental pathology, Pre-eclampsia and those without a placental cause of death (i.e. all other causes such as ascending infection, feto-maternal haemorrhage, congenital abnormalities, infection and unexplained deaths) there is a significant difference in the brain: liver weight ratio between groups ($p<0.0001$). Median 6.03 (range 0.51-10.00) versus median 4.27 (range 1.17- 9.88), median difference 1.55 (95% CI 0.86-2.21).
    a. **Finding**: SGA with placental causes of death have disproportionally lighter livers in relation to brain weight than those with birthweight $<10^{th}$ centile but no features of IUGR.
    b. **Implication**: Brain:Liver weight ratio is significantly more affected in cases of SGA with placental abnormalities, likely representing true IUGR compared to any other SGA case, which may be "physiological".

A Brain:Liver weight ratio of 6 differentiates "true" placental IUGR SGA cases from SGA cases without significant placental pathology with 53% sensitivity and 80% specificity (*Figure 69*).

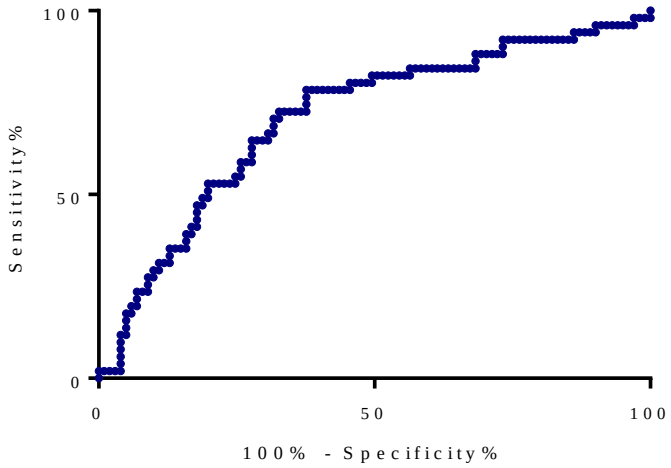


Figure 69 *ROC curve of Brain:Liver weight ratio for cases or "true" IUGR against all other SGA cases based on a brain:liver weight ratio of 6.*

Furthermore there are significant differences in the Brain:Liver weight ratios between cases of non-SGA with an unexplained cause of death, SGA with a placental causes of death (true IUGR) and SGA cases with a non-placental cause of death (p<0.0001 for all (*Figure 70).*

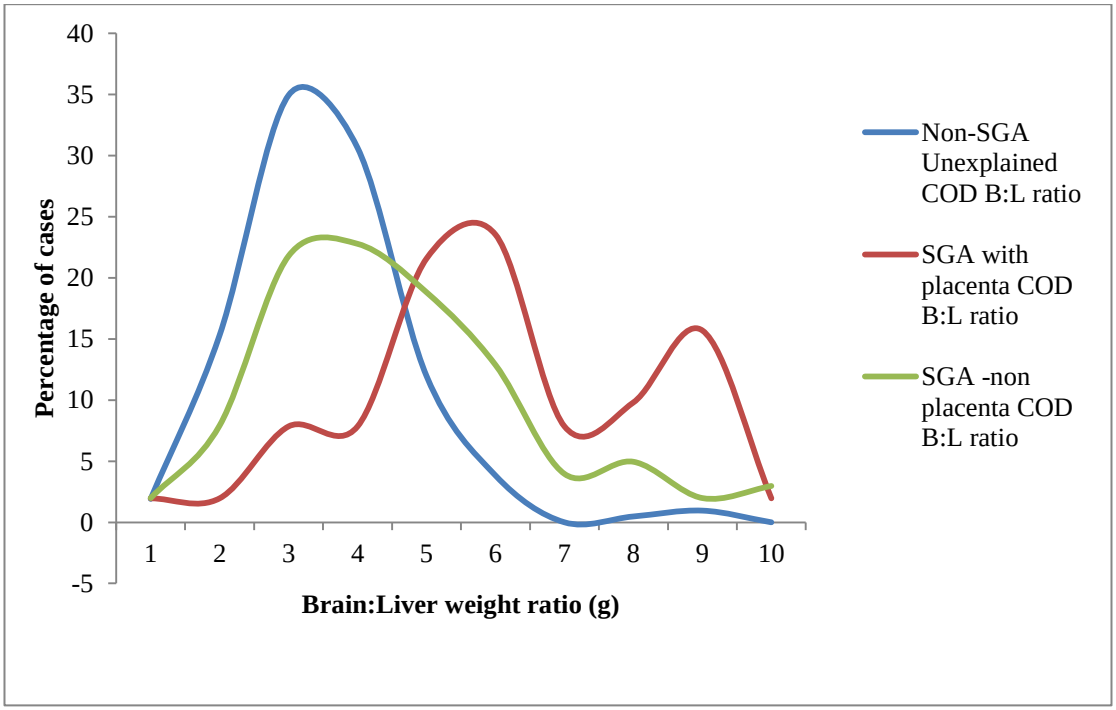


Figure 70 *Brain:Liver weight ratio in Non SGA cases with an unexplained cause of death, SGA cases with placental causes of death (true IUGR) and SGA cases with a non-placental cause of death. Each group have significantly different brain:liver weight ratios in comparison to the other (p<0.0001 for all).*

5. Brain:Liver weight ratio is significantly greater in cases of SGA with a placental cause of death compared to any other cause of death regardless of bodyweight centile (SGA and non SGA fetuses) ($p<0.0001$). Median 6.10 (range 0.51-10.00) versus median 3.59 (range 1.17- 9.88), median difference 2.38 (95% CI 1.83 – 2.94).
    a. **Finding:** SGA cases with a placental cause of death have disproportionally lighter livers to brains than cases with any other cause of death
    b. **Implication:** A greater Brain:Liver weight ratio is likely a marker of true pathological IUGR with placental abnormalities.

A Brain:Liver weight ratio of 5 would identify IUGR ("true" placental IUGR in comparison to all other causes of death) with a sensitivity of 73% and a specificity of 85%. Using a ratio of 6 would provide a specificity of 92% but would reduce the sensitivity to 55% (*Figure 71*). Note there is no Brain:Liver weight ratio that absolutely distinguishes the pathology groups; some placental IUGR cases have Brain:Liver ratio of 2 and some cases with no evidence of placental disease or other pathology have Brain:Liver ratio of 9. However, cases with birthweight> 10^{th} centile with unexplained cause of death and no placental pathological lesions only very rarely have a Brain:Liver ratio above six.

Cutoff	Sensitivity %	95% CI	Specificity %	95% CI	Likelihood ratio
> 3	91.84	80.40% to 97.73%	32.39	27.76% to 37.29%	1
> 4	83.67	70.34% to 92.68%	60.93	55.88% to 65.80%	2
> 5	73.47	58.92% to 85.05%	84.83	80.88% to 88.25%	5
> 6	55.1	40.23% to 69.33%	91.77	88.59% to 94.31%	7
> 7	30.61	18.25% to 45.42%	95.89	93.41% to 97.63%	7
> 8	24.49	13.34% to 38.87%	97.17	95.00% to 98.58%	9
> 9	6.122	1.281% to 16.87%	98.97	97.39% to 99.72%	6

Table 93 Sensitivity and specificity used to define True IUGR cases (<10th centile with evidence of placental disease) in comparison to other causes of death for Brain:Liver ratio cut offs 3-9.

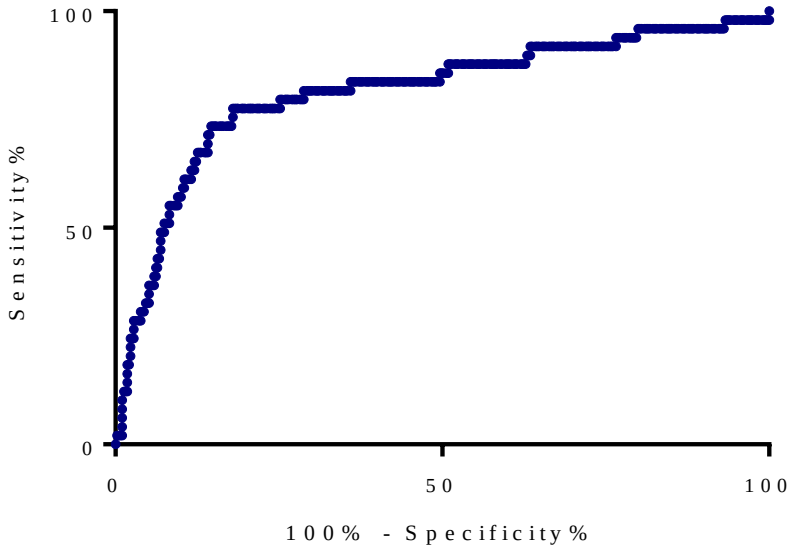


Figure 71 *ROC curve of Brain:Liver weight ratio for cases or "true" IUGR against other causes of death (SGA and non SGA) based on a Brain:Liver weight ratio of 6.*

6.4.5 Body:Thymus weight ratio

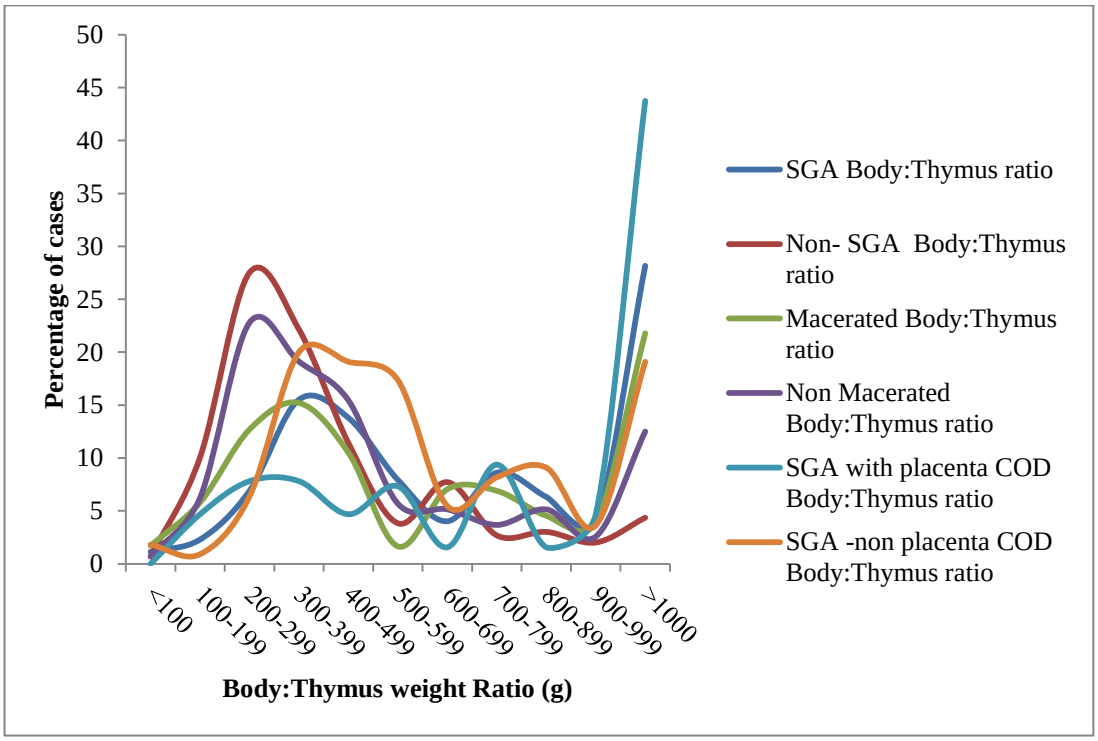


Figure 72 Proportion of cases with specific Body:Thymus weight ratios (males and females) in cases of SGA; non SGA; macerated and non-macerated; SGA cases with a placental cause of death and SGA without a placental cause of death.

Using Brain:Liver weight ratio to establish if a fetus is growth restricted requires an invasive autopsy. If Body:Thymus weight ratio could be used instead, a more limited autopsy is all that would be required.

There are significant associations between Body:Thymus weight ratio (for males and females) and fetal maceration, specific causes of death and SGA specifically:

1. Body:Thymus weight ratio is significantly greater in macerated fetuses compared to non-macerated fetuses ($p<0.0001$). Median 532.69 (range 0.005-14890) versus median 405.75 (range 4.39-13250), median difference 95.06 (95% CI 140.25 to 52.08).
    a. **Finding:** Macerated fetuses have disproportionally lighter thymus' in proportion to their body weight than non-macerated fetuses

   b. **Implication:** The thymus disproportionately loses more weight than the body in cases of maceration.

2. Body:Thymus weight ratio is significantly greater in SGA fetuses compared to non SGA fetuses ($p<0.0001$). Median 638.89 (range 5.82-14890) versus median 355.14 (range 29.39 - 2618), median difference 242.88 (95% CI 184.82 to 313.85).
   a. **Finding:** SGA fetuses have a disproportionally lighter thymus' in relation to their body weight.
   b. **Implication**: The thymus disproportionately loses more weight than the body in cases of SGA

3. If cases of SGA are divided into "true" IUGR - those with a placental cause of death (i.e. abruption, known IUGR, Placenta, Pre-eclampsia) and those without a placental cause of death (i.e. ascending infection, feto-maternal haemorrhage, congenital abnormalities, infection and unexplained deaths) there is a significant difference in the body:thymus weight ratio between groups ($p=0.0054$). Median 830.56 (range 154.38- 6016.67) versus median 551.43 (range 5.82- 14890), median difference 208.66 (95% CI 70.10 to 386.95).
   a. **Finding**: SGA with placental causes of death have disproportionally lighter thymus' in relation to body weight.
   b. **Implication**: Body:Thymus weight ratio is significantly more affected in cases of SGA with placental abnormalities than in any other SGA case, which may be "physiological".

A Body:Thymus weight ratio of 855.5 would identify "true" IUGR from other SGA cases without significant placental pathology with 50% sensitivity and 75% specificity (*Figure 73*).

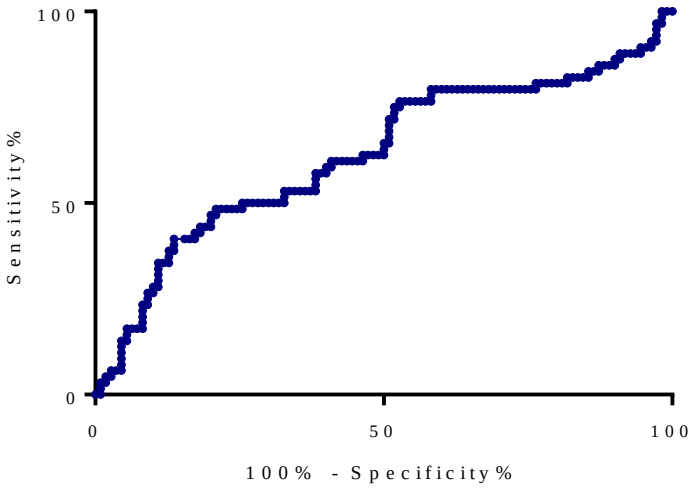


Figure 73 *ROC curve of Body:Thymus weight ratio for cases or "true" IUGR against all other SGA cases based on a Body:Thymus weight ratio of 855.5.*

4. Body:Thymus weight ratio is significantly greater in cases of SGA with a placental cause of death compared to any other causes of death regardless of bodyweight centile (SGA and non SGA fetuses) ($p<0.0001$). Median 830.56 (range 154.38- 6016.67) versus median 394.71 (range 5.82 - 14890), median difference 369.47 (95% CI 247.48 to 530.81).
    c. **Finding:** SGA cases with a placental cause of death have disproportionally lighter thymus' to body weight than cases with any other cause of death
    d. **Implication:** Body:Thymus weight ratio is related to true SGA with placental abnormalities.

A Body:Thymus weight ratio of 671.7 would identify "true" IUGR compared to all other causes of death with a sensitivity of 61% and a specificity of 79%. Using a ratio of 863.9 would provide an even higher specificity of 88% but would reduce the sensitivity to 50% (*Figure 74*).

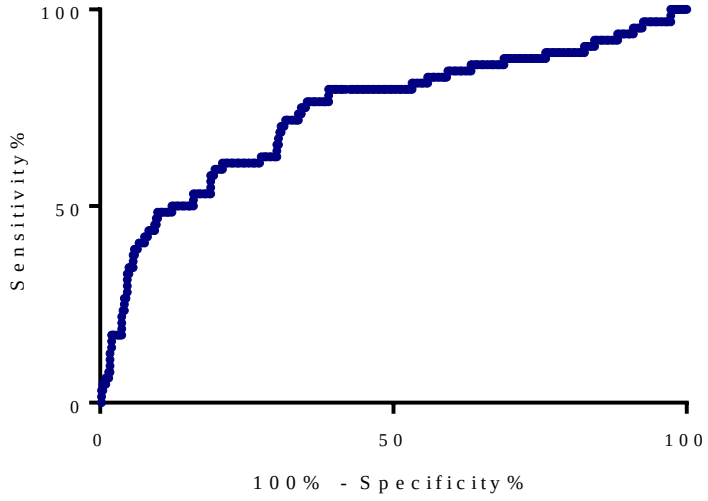


Figure 74 *ROC curve of Body:Thymus weight ratio for cases or "true" IUGR against all other causes of death (SGA and non SGA) for a Body:Thymus weight ratio of 863.9.*

Whilst abnormal, the Body:Thymus weight ratio does not identify pathological IUGR as well as the Brain:Liver weight ratio.

6.5 Discussion

The findings of this chapter have demonstrated that organ weights are significantly affected by maternal factors as well as the process of fetal maceration, the cause of death and the underlying mechanism for demise.

Firstly, diabetic mothers delivered fetuses with heavier adrenal glands than non-diabetic mothers. One other study was found reviewing autopsy data between mothers with and without Diabetes Mellitus and reported no significant difference in fetal adrenal gland weight between diabetic and non-diabetic mothers, however, the study population was much smaller than the present study(only 55 cases) (210).

The brain, liver, heart, thymus, lungs, kidneys and thyroid are all significantly lighter in SGA fetuses compared to non SGA and when taking into account body weight, the liver, spleen and thymus are significantly disproportionately lighter in cases of SGA.

The brain, liver, thymus, heart, lungs, adrenals, and thyroid are also lighter in macerated fetuses compared to non-macerated and this persists when taking into account fetal body weight, indicating preferential visceral organ weight loss with maceration.

Only the liver and thymus are significantly lighter in cases with demonstrated placental causes of death compared to other deaths and these relationships persist when taking into account fetal body weight.

As highlighted above, the overall birth weights (delta values) for fetuses with maceration were lower with a disproportionate loss of weight from almost all visceral organs. Furthermore, the liver lost more weight than the brain in macerated cases indicating that the Brain:Liver weight ratio is erroneously increased simply due to the effects of maceration rather than underlying pathology such as IUGR, and this should be accounted for when interpreting Brain:Liver weight ratios in clinical practice; on average the Brain;Liver weight ratio should be reduced by 1 unit to account for the artefactual effects of maceration. A previous, study reported similar results in which weights of the liver, thymus and spleen were markedly lighter with increasing maceration; the lungs, kidneys and adrenals moderately lighter and the heart and brain weights only slightly lighter as maceration status increased (168).

Brain:Liver weight ratio has long been used as an autopsy method of assessing fetal undernutrition and growth. However this is based on remarkably limited data. In

1972 a study of 95 stillbirths and neonatal deaths examined the association of brain to liver weight ratio, fetal gestation and fetal birthweight (169). The average Brain:Liver weight ratio in those with a mean birthweight or one standard deviation above the mean was 3 in gestations greater than 27 weeks. In those with a birthweight below the 10^{th} centile the Brain:Liver weight ratio varied at different gestations: at 25-30 weeks the ratio fell in the range of 1.7-4.1 but at 31-42 weeks 11 of 15 cases had a ratio of 4.5 or greater (169). In all of these cases the placenta was reported to show chronic insufficiency and in seven cases there was a history of pre-eclampsia, hypertension, twin birth or low urinary oestriol excretion (169).

In 2001, Brain:Liver weight ratio was analysed in 182 stillbirths with the aim of exploring the relationships between low fetal body weight and maternal history (170). Cases were split into three groups; those symmetrically small; those with asymmetrical growth restriction and those with no evidence of growth restriction (170). Growth restriction was actually SGA based on bodyweight below the 10^{th} centile. A Brain:Liver weight ratio greater than 3 was classed as abnormal and it was reported that the asymmetrically growth restricted fetuses had significantly higher Brain to Liver weight ratios ($p<0.0001$) (170). A cut off point for the Brain:Liver weight ratio of 3 had sensitivity of 55% and specificity of 63% for detecting SGA; a cut off of 6 had sensitivity of 11% but a specificity of 97% (170). If only cases greater than 20 weeks gestation were used and SGA was described as being below the 5^{th} centile rather than the 10^{th}, the sensitivity of a Brain:Liver weight ratio of 3 was 83% and the specificity 66% in detecting SGA fetuses (170).

The present results provide definitive data regarding use of Brain:Liver weight ratio in the perinatal autopsy setting. First the study is far larger than all previous studies combined, secondly, the findings are presented in relation to pathological findings in

addition to simple birthweight centiles, and finally, data is presented on the impact of changes such as maceration, which were not evaluated in any previous study. The present study data confirms that the Brain:Liver weight ratio is significantly greater in cases of SGA with a placental cause of death compared to cases with birthweight < 10^{th} centile but with no pathological placental findings or with another cause of death. A Brain:Liver weight ratio of 5 can identify "pathological" placental IUGR compared to other causes of death with a sensitivity of 73% and a specificity of 85%. A ratio of 6 improves specificity to 92% but reduces sensitivity to 55%. Furthermore, it is possible to differentiate "pathological" IUGR (i.e. cases of SGA with concurrent placental abnormalities and a placental cause of death) from cases that have biometry below the 10^{th} centile but with no placental abnormalities using a Brain:Liver weight ratio of 6 for a sensitivity of 53% and a specificity of 80%. In addition, since for all causes of death cases with maceration have artefactually relatively lighter livers secondary to the process occurring after death, on average, it is necessary to adjust Brain:Liver weight ratio by around 1 unit in macerated fetuses to avoid erroneously assigning IUGR where non exists.

Only one study to date has evaluated the accuracy of postmortem magnetic resonance imaging (MRI) in the measurement of fetal organ volumes compared to the standard autopsy measurements of organ weights (213). This MRI study was small; 25 perinatal deaths from 16-40 weeks gestation were examined (213). MRI was performed prior to autopsy and it was found that there was a linear relationship between MRI organ volume estimates and the autopsy organ weights recorded (214). ROC curve statistical analysis demonstrated an area of 0.61 for postmortem MRI detection of a Brain:Liver weight ratio greater than or equal to 4 (214). This suggests

that postmortem MRI may have the potential to replace invasive autopsy measurements of Brain:Liver weight ratio but further larger studies are needed.

The present study also evaluated whether a more limited autopsy approach that does not involve removal of the brain, could provide accurate assessment of fetal growth restriction by examining the relationship between the Body:Thymus weight ratio and fetal maceration, fetal SGA and cause of death. Results showed significant differences between macerated and non-macerated groups; SGA and non SGA groups; and SGA cases with and without placental causes of death. However, although useful, the sensitivity and specificity of Body:Thymus weight ratios are not as effective as brain to liver weight ratio for detecting "true" IUGR.

This study is the first large scale, detailed evaluation of fetal organ weights in stillbirths and has determined that fetal maceration and cause of death affect specific organ weights. More importantly the study has evaluated, in detail, the role of the Brain:Liver weight ratio in the pathological detection of IUGR/ SGA. In conclusion, the Brain:Liver weight ratio, when calculated in the correct context and taking into account fetal maceration, may be used to help evaluate and separate cases of "true" fetal IUGR and SGA from more physiological cause of SGA in stillbirth autopsy.

7. The Placenta

7.0 Background

7.1 Methods

7.2 Results

- 7.2.1 Cord insertion
- 7.2.2 Maternal Ethnicity
- 7.2.3 Histology of the cord, membranes and placenta and cause of death
- 7.2.4 Ascending Infection
- 7.2.5 Maternal Vascular malperfusion
- 7.2.6 Other significant placental pathology
- 7.2.7 Unexplained placental lesions

7.3 Discussion

7.0 Background

The placenta and the umbilical cord are the life support system for a human fetus. Abnormalities and infections within the arteries, veins, villi or umbilical cord can fatally disrupt blood and nutrient flow to the developing fetus. (117)

Abnormalities that can occur include:

1. Ascending Genital Tract infections which can cause preterm delivery, hypoxia and death (142).
2. Haematogenous Infections such as Toxoplasmosis and cytomegalovirus (144)
3. Hypertension and Pre-eclampsia/Eclampsia of which the former affects around 5-8% of pregnancies (145). Pre-eclampsia can be defined as maternal endothelial dysfunction with clinical hypertension, oedema and proteinuria (117).
4. Placental Abruption, defined as the complete or partial separation of a normally implanted placenta before delivery (148). Complications may include fetal death, severe haemorrhage, need for blood transfusions, emergency hysterectomy, disseminated intravascular coagulopathy and renal failure (148).
5. Specific pathologies only diagnosable on histological examination associated with growth restriction and fetal death such as massive perivillous fibrin deposition (149)
6. The spectrum of changes associated with maternal vascular malperfusion including those previously known as uteroplacental vascular disease (150).

Placental abnormalities are a common cause of death in stillbirth, ranking second only to unexplained deaths (151). A review of over 40 papers of placental pathology

in association with stillbirth reported that the placenta was the likely cause of stillbirth in 11 - 65% of cases, with placental abruption the most frequent specific cause (152). However, at present there are no clinically useful first or second-trimester tests of placental function to predict stillbirth, although uterine artery Doppler indices and maternal serum PAPP-A levels appear to be promising candidates for early detection of placental dysfunction (153).

The aim of this chapter is to analyse all placentas submitted for examination in stillbirth and miscarriage autopsies in relation to:

1. Placental cord insertion
2. Placental histological findings and their relationship to cause of death
3. Detailed analysis of placentas with ascending infection or maternal vascular malperfusion with regard to gestational age, type of death, maceration and maternal demographic factors.
4. Detailed review of cases with other specific significant placental pathologies.
5. Review of cases with unexplained cause of death and placental lesions of uncertain significance.

7.1 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriage from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Data was analysed through queries and statistical tests run using Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Statistical tests can be viewed in detail in Appendix 3.

7.2 Results

946 of 1,064 (89%) total cases had placentas submitted for examination as part of the autopsy (*Table 94*)

	Early miscarriage	Late miscarriage	Stillbirth	**Total**
Number of cases with a placenta submitted	203 (83%)	168 (94%)	575 (90%)	**946 (89%)**
Number of cases with no placenta submitted	43 (17%)	11 (6%)	64 (10%)	**118 (11%)**
Total:	**246**	**179**	**639**	**1064**

Table 94 Placenta submitted for examination within each death category

7.2.1 Maternal Ethnicity and the Placenta

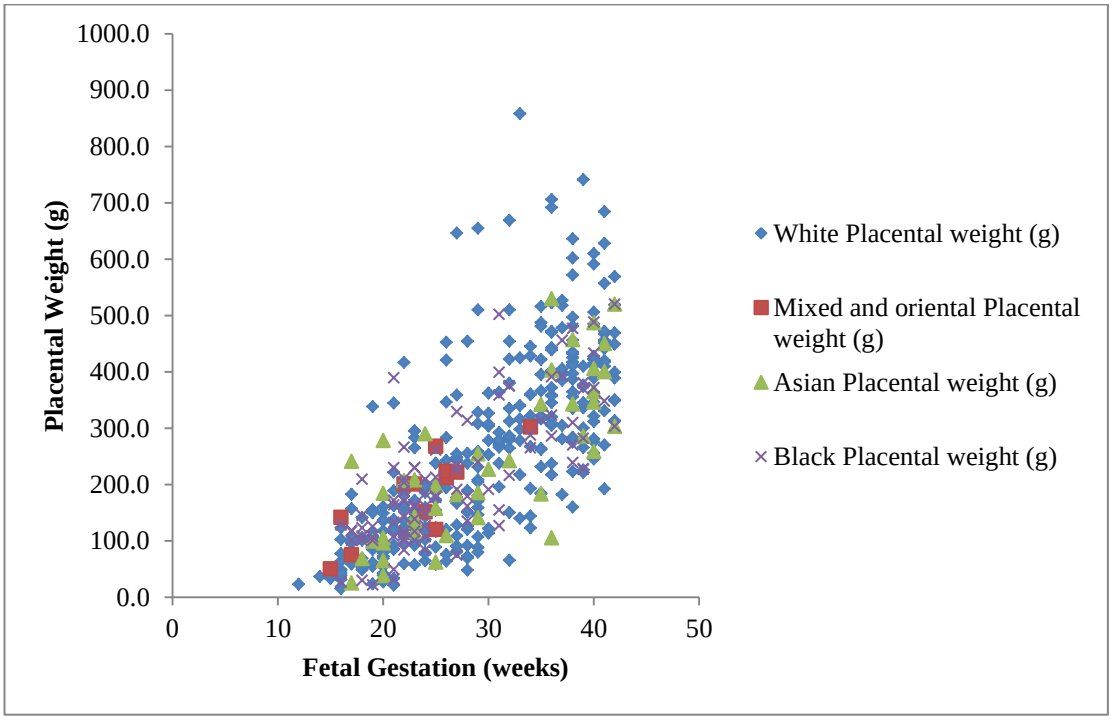


Figure 75 *Placental disc weight and maternal ethnicity*

There was a recorded maternal ethnicity in 403 of the 946 placentas submitted for examination including:

- 337 white mothers

- 107 black mothers
- 44 Asian mothers
- 12 mixed or oriental mothers

There was a significant difference in the placental weight between black and white mothers: white mothers had significantly heavier placentas than black mothers (p= 0.014). However the placental weight was not adjusted for gestational age and it is known that black mothers had more frequent miscarriages than white mothers (Chapter 3) and this is therefore the likely cause for this difference.

7.2.2 Cord Insertion

Overall, the cord was most frequently eccentrically inserted into the placenta and there were no significant differences in cord insertion site between categories (insertion site based on description of reporting pathologist at the time of autopsy).

Cord insertion	Early miscarriage	Late miscarriage	Stillbirth	**Total**
Not given	119 (48%)	54 (30%)	141 (22%)	**314 (29%)**
Central	46 (19%)	32 (18%)	174 (27%)	**252 (24%)**
Eccentric	69 (28%)	74 (41%)	255 (40%)	**398 (37%)**
Marginal	8 (3%)	13 (7%)	42 (7%)	**63 (6%)**
Vellamentous	4 (2%)	5 (3%)	24 (4%)	**33(3%)**
Furcate	0 (0%)	0 (0%)	2 (<1%)	**2 (<1%)**
Other	0 (0%)	1 (1%)	1 (<1%)	**2 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 95 Cord insertions in each death category

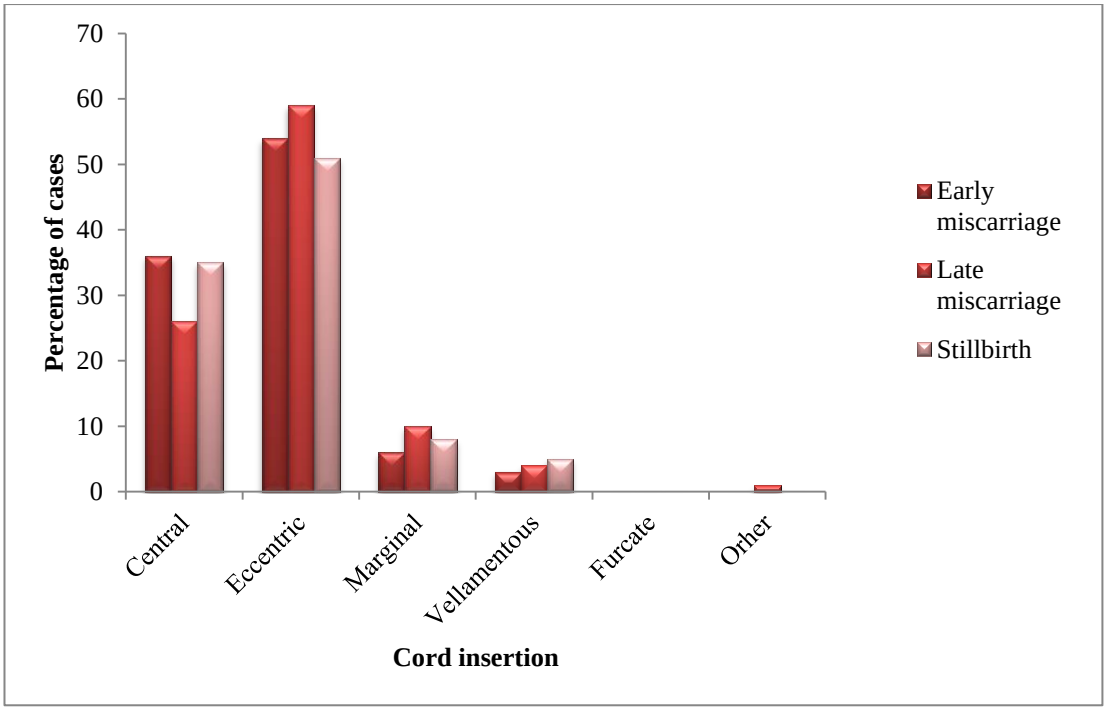


Figure 76 *Cord insertions for each death category (excluding not givens)*

A large UK study recorded the cord insertion in 861 singleton pregnancies live born at 37-42 weeks gestation. The study reported a cord centrality index (CI) – a measurement used to assess the point of umbilical cord insertion from the centre of the placenta. The CI ranged from 0.02 – 1.0 with a mean of 0.36 and was not significantly different between affected (i.e. cases with pre-eclampsia, pregnancy induced hypertension, Diabetes or SGA) and non-affected cases. When comparing results to the present study, it was found that the stillbirth population in the present study had significantly more central and vellementous cord insertions and live born fetuses had significantly more eccentric cord insertions ($p= 0.0116$, $p<0.0001$ and $p<0.0001$ respectively) (*Table 96*) but these are very subjective categories.

Cord insertion	Study Population	Reference Live born population
Central	252 (34%)	239 (28%)
Eccentric	398 (53%)	551 (64%)
Furcate	2 (<1%)	0 (0%)
Marginal	63 (8%)	69 (8%)
Vellementous	34 (5%)	2 (<1%)
Other	2 (<1%)	0 (0%)
Total:	**751**	**861**

Table 96 Cord insertions in present study and reference study population. The stillbirth population in the present study had significantly more central and vellementous cord insertions and live born fetuses had significantly more eccentric cord insertions (p= 0.0116, p<0.0001 and p<0.0001 respectively)

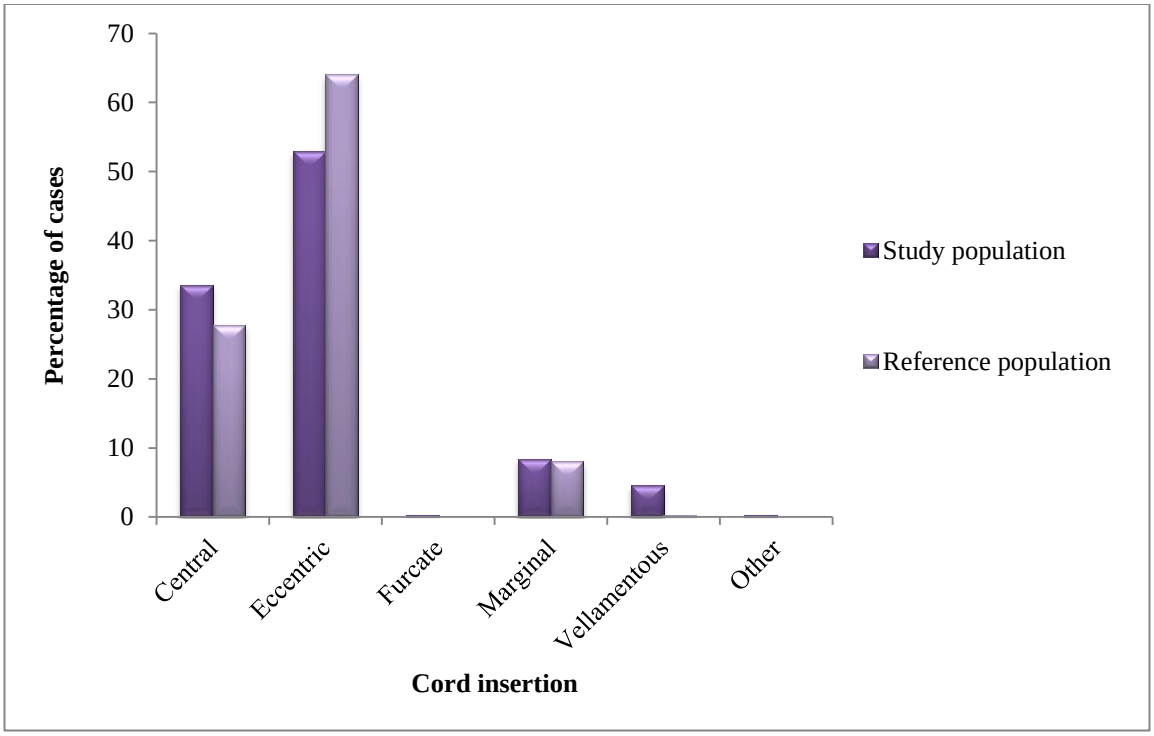


Figure 77 *Cord insertions in present study and reference study population. The stillbirth population in the present study had significantly more central and vellementous cord insertions and live born fetuses had significantly more eccentric cord insertions (p= 0.0116, p<0.0001 and p<0.0001 respectively)*

7.2.3 Histology of the cord, membranes and placenta and cause of death

Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Cord, membranes and placenta normal	94 (38%)	70 (39%)	172 (27%)	**336 (32%)**
Cord and placenta normal, membranes abnormal	9 (4%)	9 (5%)	28 (4%)	**46 (4%)**
Cord normal, membranes and placental abnormal	32 (13%)	17 (9%)	68 (11%)	**117 (11%)**
Cord and membranes normal, placenta abnormal	38 (15%)	31 (17%)	196 (31%)	**265 (25%)**
Cord abnormal, membranes and placental normal	1 (1%)	2 (1%)	17 (3%)	**20 (2%)**
Cord and membranes abnormal, placental normal	1 (1%)	10 (6%)	5 (1%)	**16 (2%)**
Cord, membranes and placenta abnormal	19 (8%)	22 (12%)	49 (8%)	**90 (8%)**
Cord and placenta abnormal, membranes normal	4 (2%)	4 (2%)	33 (5%)	**41 (4%)**
Not information given	38 (15%)	13 (7%)	57 (9%)	**108 (10%)**
Too Autolysed	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Cord Normal, no other histology	4 (2%)	0 (0%)	10 (2%)	**14 (1%)**
Cord Abnormal, no other histology	2 (1%)	0 (0%)	2 (<1%)	**4 (<1%)**
No cord, placenta normal	0 (0%)	1(1%)	1 (<1%)	**31 (3%)**
No cord, membranes and placenta abnormal	3 (1%)	0 (0%)	1 (<1%)	**4 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 97 Histological findings of the cord, membranes and placenta in each death category.

One third of all deaths had completely normal histology of the cord, membranes and placenta. However, nearly one third of all stillbirths had at least some abnormality

identified on histological examination of the placenta (with normal membranes and cord); a significantly greater proportion than in cases of miscarriage ($z=5.689$, $p<0.0001$). 347 stillbirths had at least some degree of abnormal placental histology (not including any abnormalities of the cord or membranes) of which 208 had significant abnormalities that either were a direct cause of death (labelled as placenta or abruption- as per definitions in Chapter 4) or may have been indirectly linked to the cause of death (for example ascending infection, twin complications or maternal infection) (*Table 98*).

Specific cause of death	Number of cases with a placental abnormality linked to cause of death
Abruption	26 (13%)
Ascending Infection	49 (24%)
Placenta	48 (23%)
Unexplained placenta	50 (24%)
Pre-eclampsia	14 (7%)
Twin complications	8 (4%)
Known IUGR	4 (2%)
Fetomaternal haemorrhage	5 (2%)
Maternal infection	4 (2%)
Total:	**208**

Table 98 Specific causes of death diagnosed by abnormalities in the histological examination of the placenta (definitions for causes can be found in Chapter 4)

Overall (including stillbirths and miscarriages), there were 304 cases in which abnormalities of the placenta (found histologically or through a good clinical history)

provided a specific cause of death; 58% were cases of ascending infection, mainly associated with miscarriage (66%) (*Table 99, Figure 78).*

Causes of death provided by histological abnormalities of the placenta	Miscarriages	Stillbirths	**Total**
Abruption	8 (21%)	30 (79%)	**38 (13%)**
Ascending Infection	117 (66%)	59 (34%)	**176 (58%)**
Known IUGR	9 (50%)	9 (50%)	**18 (6%)**
Placenta *	5 (9%)	51 (91%)	**56 (18%)**
Pre-eclampsia	0 (0%)	16 (100%)	**16 (5%)**
Total	**139 (46%)**	**165 (54%)**	**304**

Table 99 Specific causes of death provided by histological abnormalities of the placenta

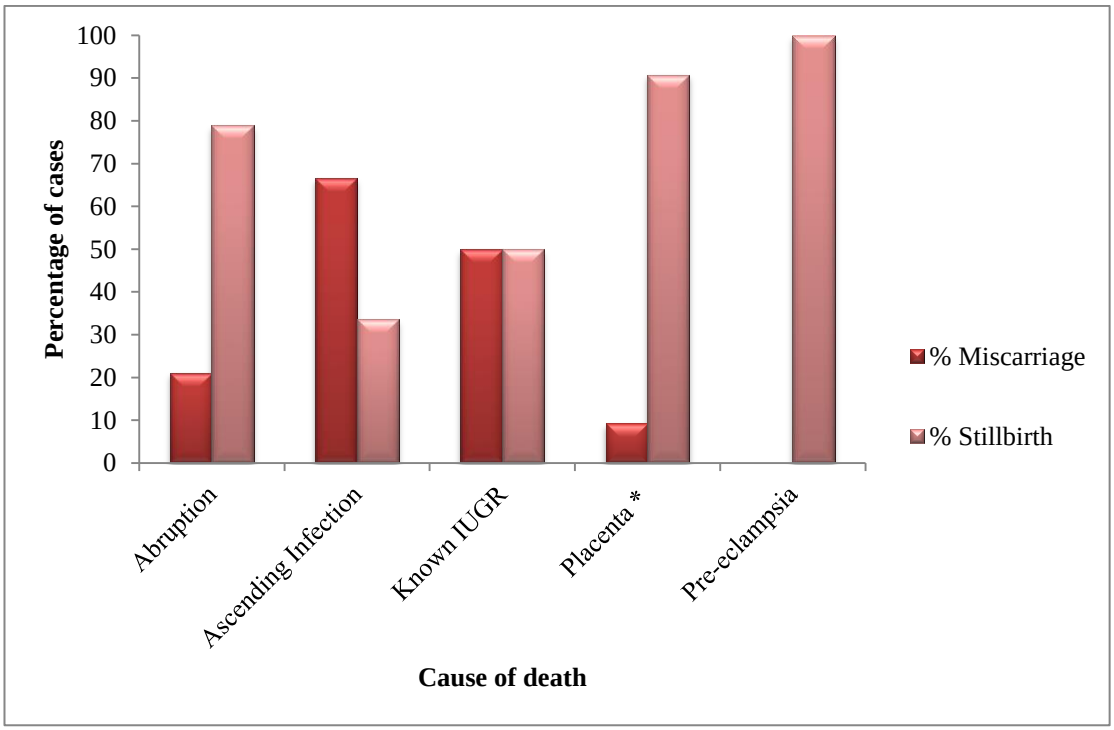


Figure 78 *Specific causes of death provided by histological abnormalities of the placenta*

18% of all causes of death* (*Table 99)* were from specific placental abnormalities labelled "placenta" (*Table 100*)

*Placental Pathology in "Placenta" cause of death:	Miscarriages	Stillbirths	**Total:**
Fetal Vascular Occlusion	0 (0%)	5 (100%)	**5 (9%)**
Chronic Histiocytic intervillositis	1 (33%)	2 (67%)	**3 (5%)**
Massive perivillous fibrin deposition	1 (17%)	5 (83%)	**6 (11%)**
Maternal vascular malperfusion	3 (7%)	39 (93%)	**42 (75%)**
Total:	**5 (9%)**	**51 (91%)**	**56**

Table 100 Significant placental pathology in cases with "placenta" as their cause of death

The majority of cases (91%) with specific placental abnormalities were in stillbirths and overall the most common pathological abnormality was maternal vascular malperfusion, accounting for 75% of cases.

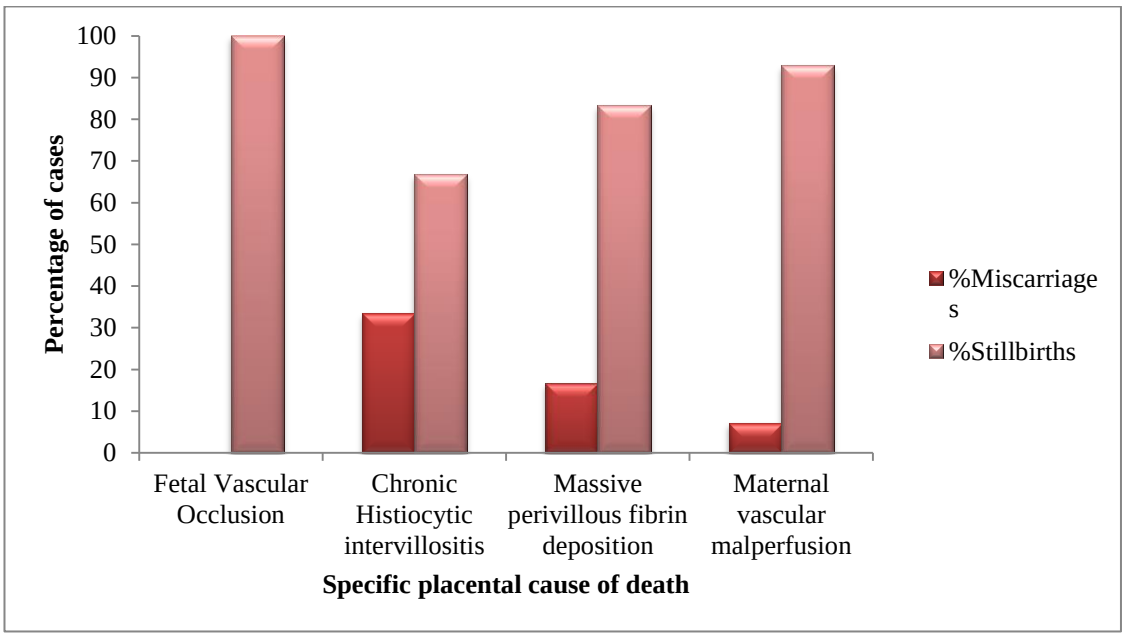


Figure 79 *Significant placental pathology in cases with "placenta" as their cause of death*

7.2.4 Ascending infection

176 autopsies were given the diagnosis of ascending infection as a cause of death with histological evidence of chorioamnionitis within the placenta, membranes or cord and/ or fetal pneumonia. 165 of these cases had placentas submitted for histological examination. Cases of ascending infection peaked in fetuses miscarried / stillborn at 22 weeks gestation (*Figure 80*). There was a further peak at term/post-term. In such cases determining whether the ascending infection was a cause or contributor to death with certainty is impossible.

Gestation (weeks)	Number of cases
13	1 (1%)
14	1 (1%)
15	2 (1%)
16	2 (1%)
17	14 (8%)
18	17 (10%)
19	13 (8%)
20	12 (7%)
21	12 (7%)
22	24 (14%)
23	18 (11%)
24	8 (5%)
25	9 (5%)
26	0 (0%)
27	2 (1%)
28	3 (2%)
29	1 (1%)
30	1 (1%)
31	0 (0%)
32	3 (2%)
33	3 (2%)
34	0 (0%)
35	1 (1%)
36	4 (2%)
37	0 (0%)
38	0 (0%)
39	1 (1%)
40	4 (2%)
>40	12 (7%)
Total:	**169**

Table 101 Number and percentage of ascending infection cases with different fetal gestations (excludes 7 cases with unknown gestation)

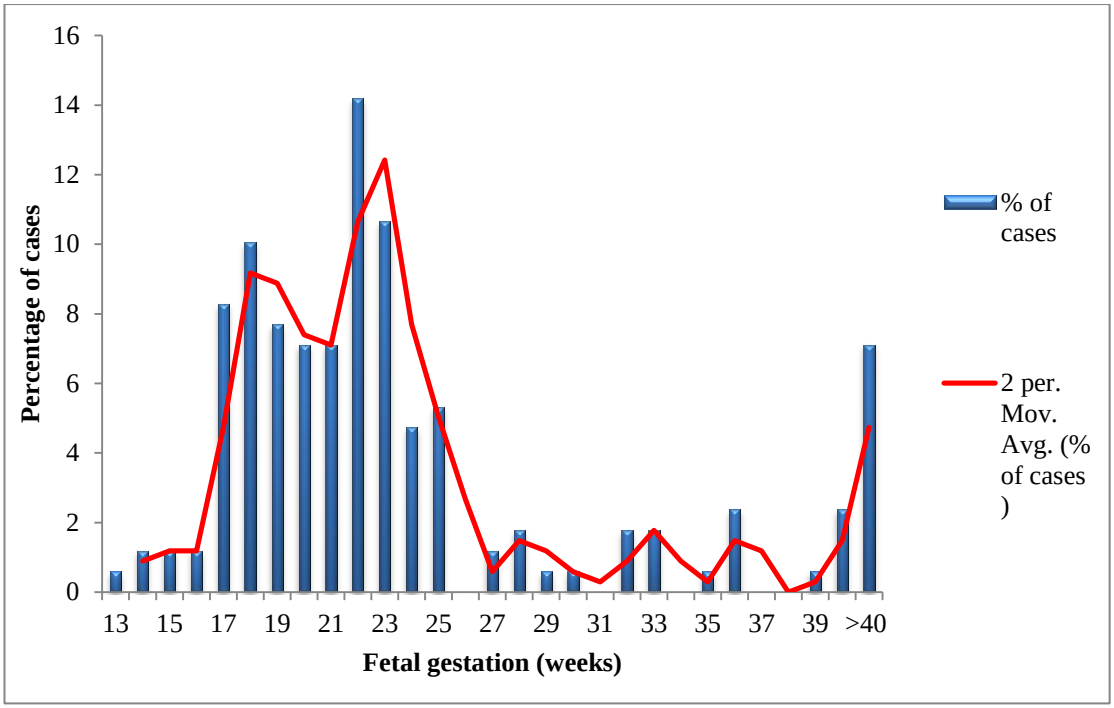


Figure 80 *Fetal gestation in cases of ascending infection (excludes 4 cases with no gestation)*

Type of death

Type of death	Number of cases
Intrauterine death < 24 hours retention	31 (18%)
Intrauterine death > 24 hours retention	27 (15%)
Intrapartum death - known	9 (5%)
Intrapartum death - fresh	109 (62%)
Total:	**176**

Table 102 Number and percentage of cases of each death type in those foetuses with ascending infection as their cause of death

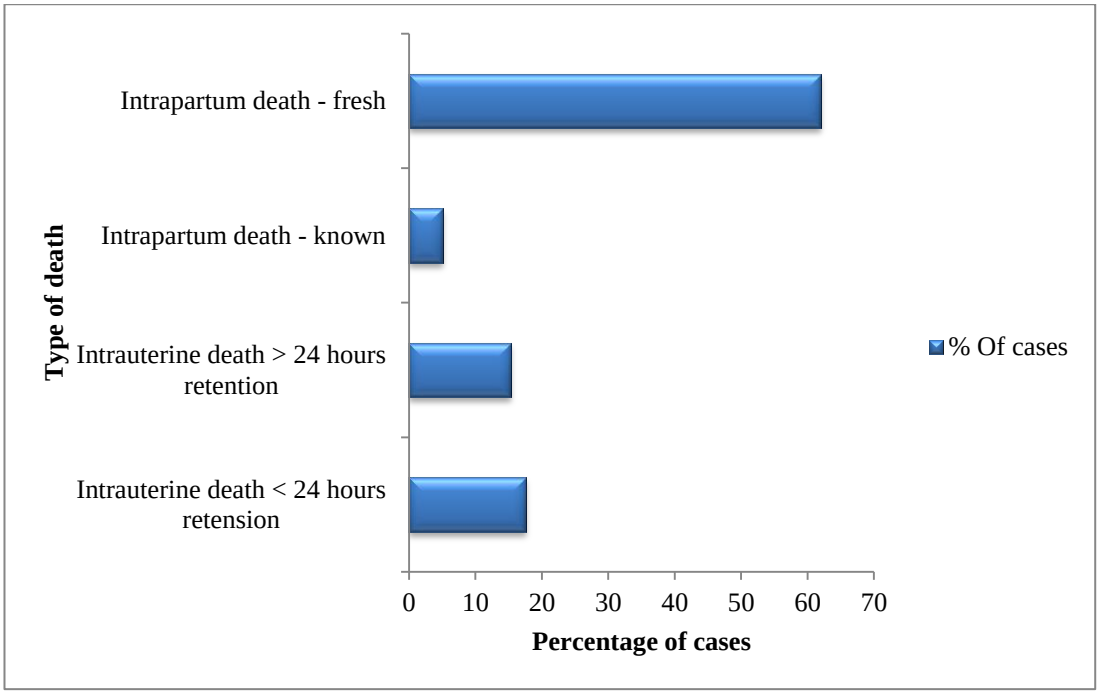


Figure 81 *Percentage of cases with ascending infection in each death type*

The majority of fetuses with ascending infection (62%) were fresh intrapartum deaths, followed by intrauterine death with an intrauterine retention time of less than 24hours (18%).

Of those cases with ascending infection and intrauterine retention of more than 24 hours (27 cases), the majority (46%) were delivered within 2 days of intrauterine death (*Table 103)* and five cases had a record of premature rupture of membranes in the antenatal history provided. The cases with longer intrauterine intervals are likely to represent secondary ascending infection rather than infection as the underlying cause of death.

Intrauterine Interval (days)	Number of cases
2	6 (46%)
3	2 (15%)
4	0 (0%)
5	2 (15%)
6	0 (0%)
7	1 (7%)
>10	1 (7%)
>20	1 (7%)
Total:	**13**

Table 103 Intrauterine interval in those cases with intrauterine retention>24 hours (14 cases had no recordable intrauterine interval)

Fetal Maceration

Fetal Maceration	Number of cases
None	125 (72%)
Mild	22 (13%)
Moderate	5 (3%)
Severe	16 (9%)
Other	6 (3%)
Total	**174**

Table 104 Degree of maceration in cases of ascending infection (2 cases excluded as no details of maceration given)

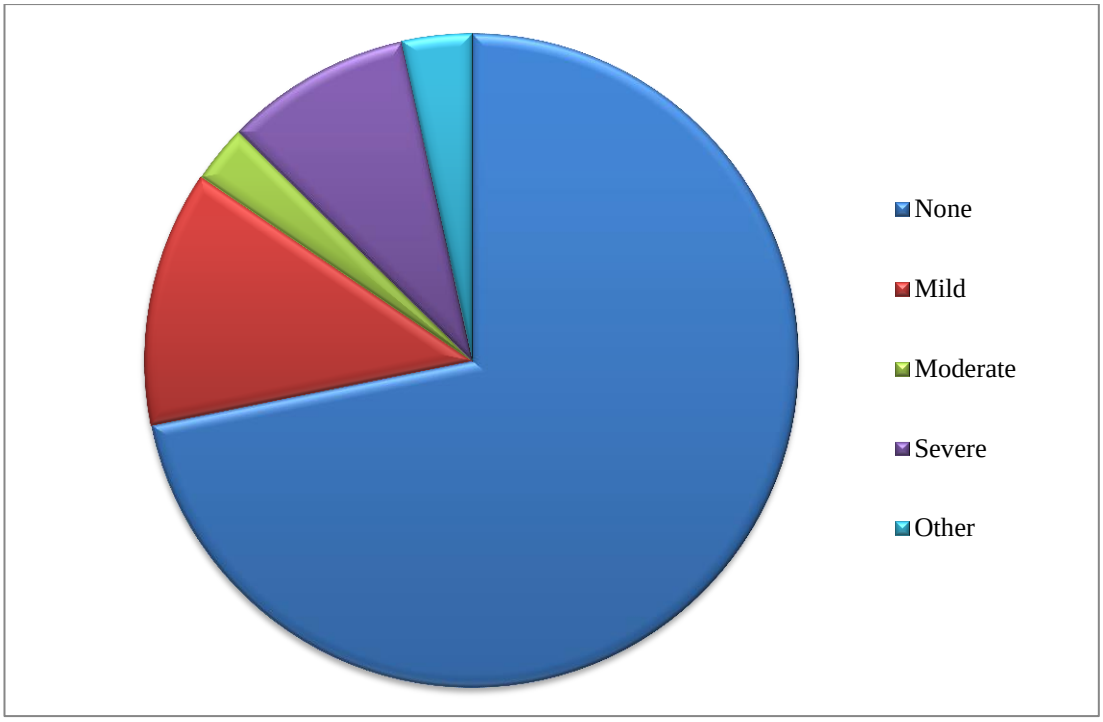


Figure 82 *Degree of maceration in cases of ascending infection*

The majority of cases had no or mild fetal maceration in keeping with the fact most cases were fresh intrapartum deaths as a consequence of the ascending infection. Of those with moderate maceration;

- Four cases were intrauterine deaths of >24 hours
- One case was an intrauterine death of < 24 hours.

In the 16 cases with severe maceration, the majority (14 cases) were retained in utero for > 24 hours.

Maternal ethnicity

Maternal ethnicity	Number of cases with Ascending Infection	Number of cases with different cause of death	**Total**
White	48 (36%)	421 (68%)	**469**
Mixed or Chinese	3 (2%)	15 (2%)	**18**
Asian	12 (9%)	46 (7%)	**58**
Black	69 (52%)	137 (22%)	**206**
Total	**132**	**619**	**751**

Table 105 Cases of ascending infection by maternal ethnicity

Black mothers delivered a significantly greater proportion of fetuses with ascending infection than any other ethnicity (z=7.046, p =<0.0001) and were 4.3 times more likely to have a case of ascending infection than white mothers (p<0.0001)

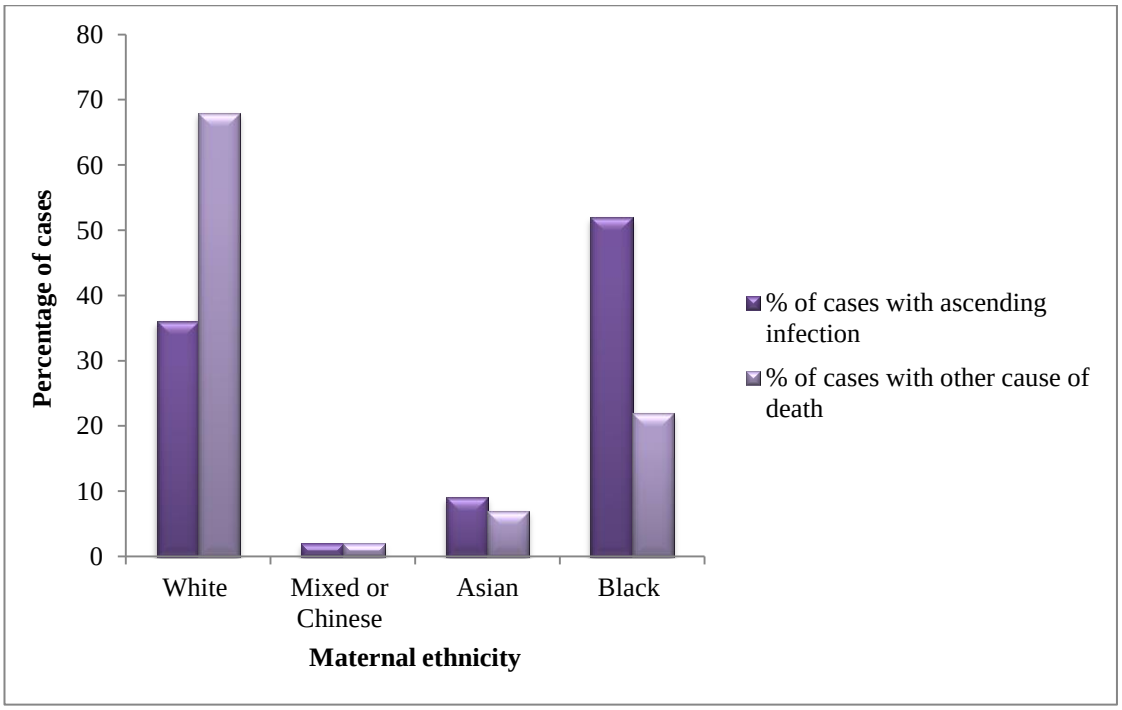


Figure 83 *Maternal ethnicity in cases of ascending infection and all other causes of death*

Maternal Age

Maternal Age (years)	Number of cases with ascending Infection	Number of cases with different cause of death	**Total**
Less than or equal to 35	130 (75%)	40 (61%)	**170**
36-40	36 (21%)	24 (36%)	**60**
>40	8 (5%)	2 (3%)	**10**
Total:	**174**	**66**	**240**

Table 106 Maternal age in cases with and without ascending infection

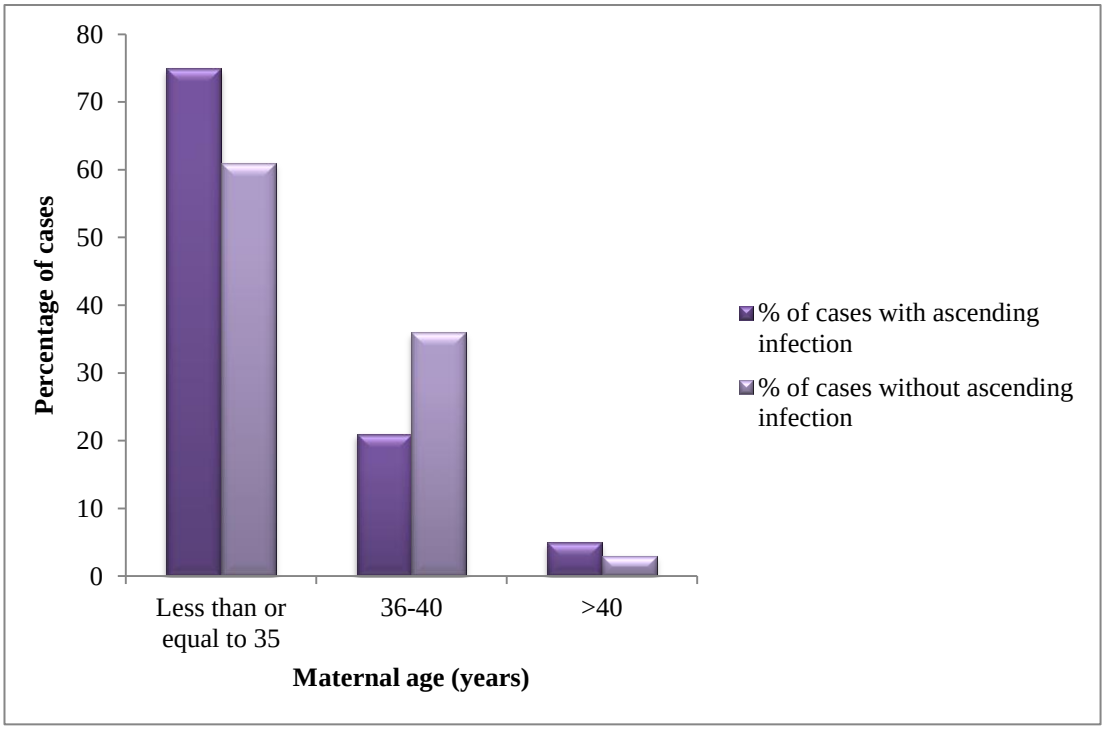


Figure 84 *Cases with ascending infection by maternal age groups*

The majority of mothers had an age of equal to or less than 35 years. There was no significant difference in the maternal age distribution between cases of ascending infection and all other deaths in the study population.

Maternal BMI

Maternal BMI	Number of cases with ascending infection	Number of cases with a different cause of death	**Total**
<18	0 (0%)	16 (4%)	**16 (3%)**
20-24	30 (35%)	112 (29%)	**142 (30%)**
25-30	30 (35%)	132 (35%)	**162 (35%)**
30+	25 (29%)	121 (32%)	**146 (31%)**
Total:	**85**	**381**	**466**

Table 107 Maternal BMI distribution for cases of ascending infection (91 cases excluded as no BMI given)

In cases of ascending infection, there was an equal proportion of mothers with a normal and an overweight BMI and there was no significant difference in the BMIs of mothers who delivered fetuses with ascending infection compared to all other causes of death at autopsy.

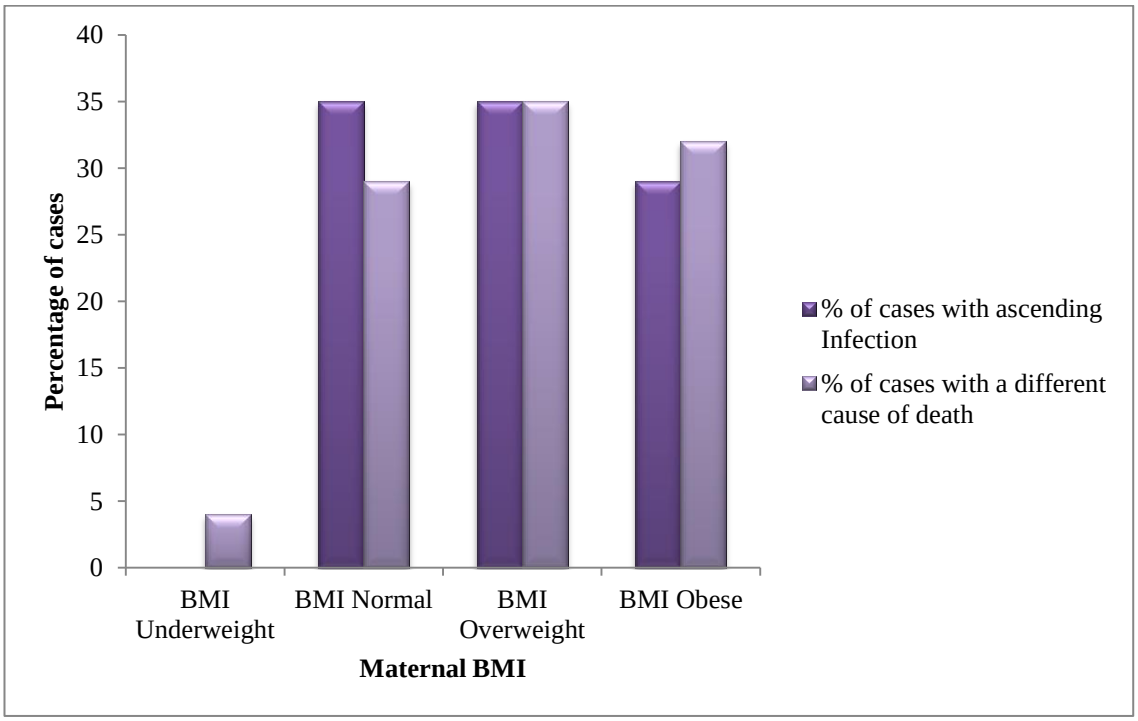


Figure 85 *Maternal BMI in cases with and without ascending infection*

Summary

In cases of ascending infection:

- The majority of cases were fresh intrapartum deaths and as such had no maceration, the death as a consequence of the ascending infection and severe preterm labour.
- There were two gestational age peaks; one large at 22 weeks and another smaller at 40+ weeks.
- Black mothers were significantly more likely to have deaths associated with ascending infection with an odds ratio of 4.3 compared to white mothers.
- There was no association between maternal age or BMI and rates of ascending infection compared to other causes of death

7.2.5 Maternal vascular malperfusion

Fetal Gestation

Gestation (weeks)	Number/Percentage of cases
23	1 (2%)
24	7 (17%)
25	1 (2%)
26	2 (5%)
27	2 (5%)
28	5 (12%)
29	5 (12%)
30	2 (5%)
31	2 (5%)
32	2 (5%)
33	1 (2%)
34	2 (5%)
37	2 (5%)
38	3 (7%)
39	2 (5%)
40	2 (5%)
42	1 (2%)
Total:	**42**

Table 108 Fetal gestations in cases of maternal vascular malperfusion

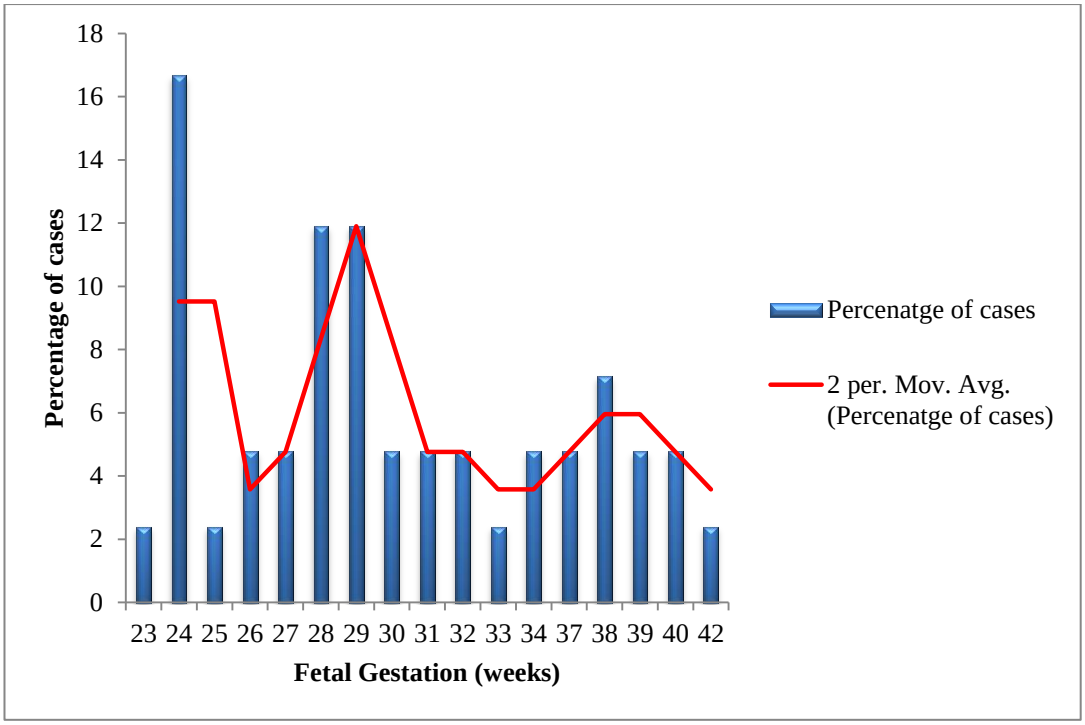


Figure 86 *Fetal gestation in cases of maternal vascular malperfusion*

There were two peaks of cases with histological evidence of maternal vascular malperfusion at 24 weeks gestation and a smaller peak at 28-29 weeks gestation. The frequency of typical maternal vascular malperfusion in term stillbirths (37+ weeks) was only 24%.

Type of Death

90% of maternal vascular malperfusion cases were intrauterine deaths with an intrauterine retention time of at least 24 hours (*Table* 109)

Type of death	Number/ percentage of cases
Intrauterine death < 24 hours retension	4 (10%)
Intrauterine death > 24 hours retention	38 (90%)
Intrapartum death – known	0 (0%)
Intrapartum death – fresh	0 (0%)
Total:	**42**

Table 109 Type of death in cases of maternal vascular malperfusion

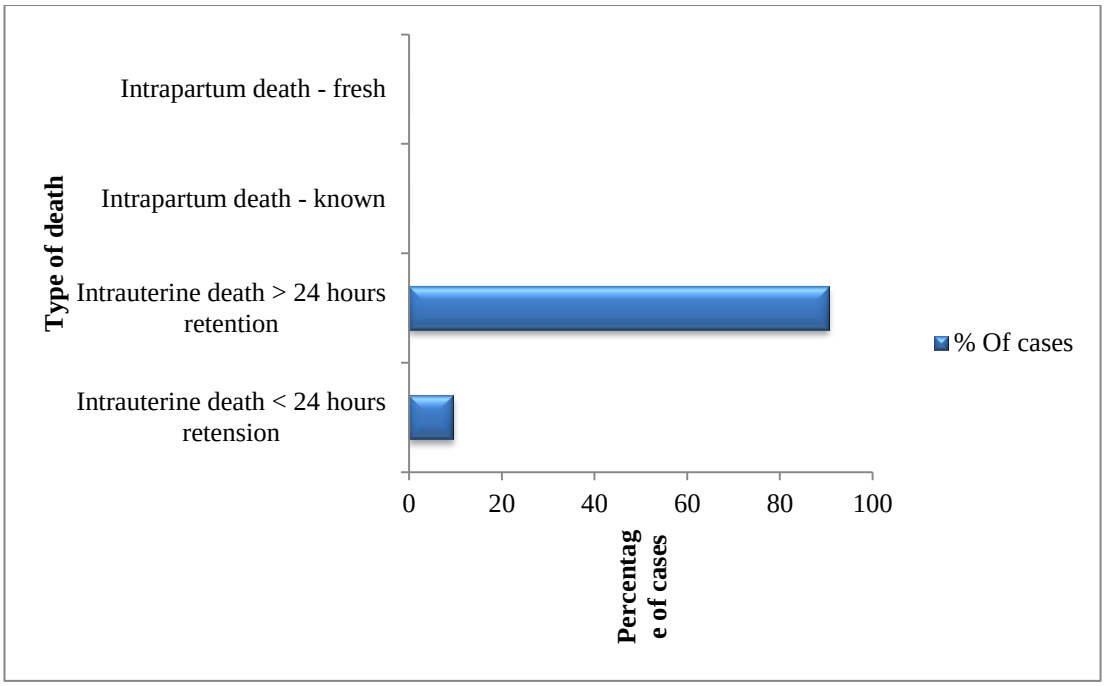


Figure 87 *Type of death in cases of maternal vascular malperfusion*

Fetal Maceration

Fetal Maceration:	Number of cases
None	1(2%)
Mild	4 (10%)
Moderate	6 (15%)
Severe	26 (63%)
Other description	4 (10%)
Total: (excluding not givens)	**41**
Not given	1

Table 110 Degree of fetal maceration in cases of maternal vascular malperfusion

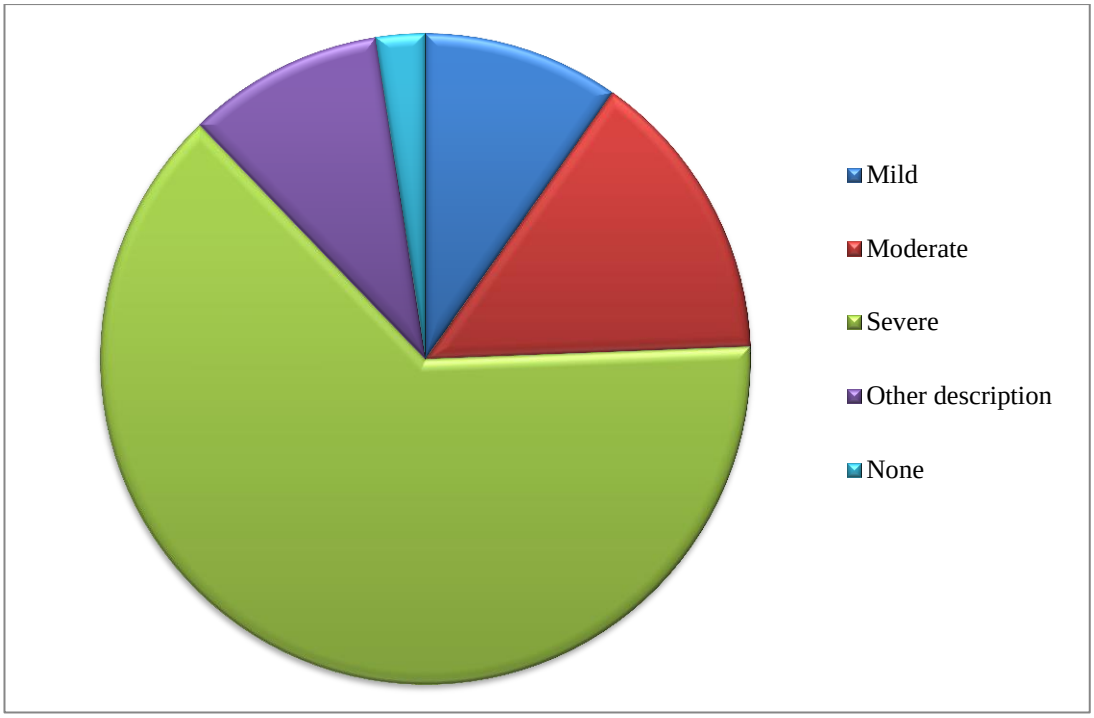


Figure 88 *Degree of fetal maceration in cases of maternal vascular malperfusion*

98% of fetuses with maternal vascular malperfusion had some degree of maceration, with the majority being severely macerated; a finding in keeping with the fact the most cases had a prolonged intrauterine interval, death occurring in the absence of labour or other maternal symptoms.

Maternal Ethnicity

Mums Ethnic Group	Number of cases with maternal vascular malperfusion	Number of cases with a different cause of death	Total
White	27 (82%)	442 (63%)	469 (64%)
Asian/Asian British Indian	1 (3%)	57 (8%)	58 (8%)
Black	5 (15%)	201 (29%)	206 (28%)
Total:	**33**	**700**	**733**

Table 111 Maternal ethnicity in cases of maternal vascular malperfusion (excluding 9 unknowns) and other causes of death

There were significantly more white mothers with a diagnosis of maternal vascular malperfusion than any other ethnicity (z=2.377, p=0.0175).

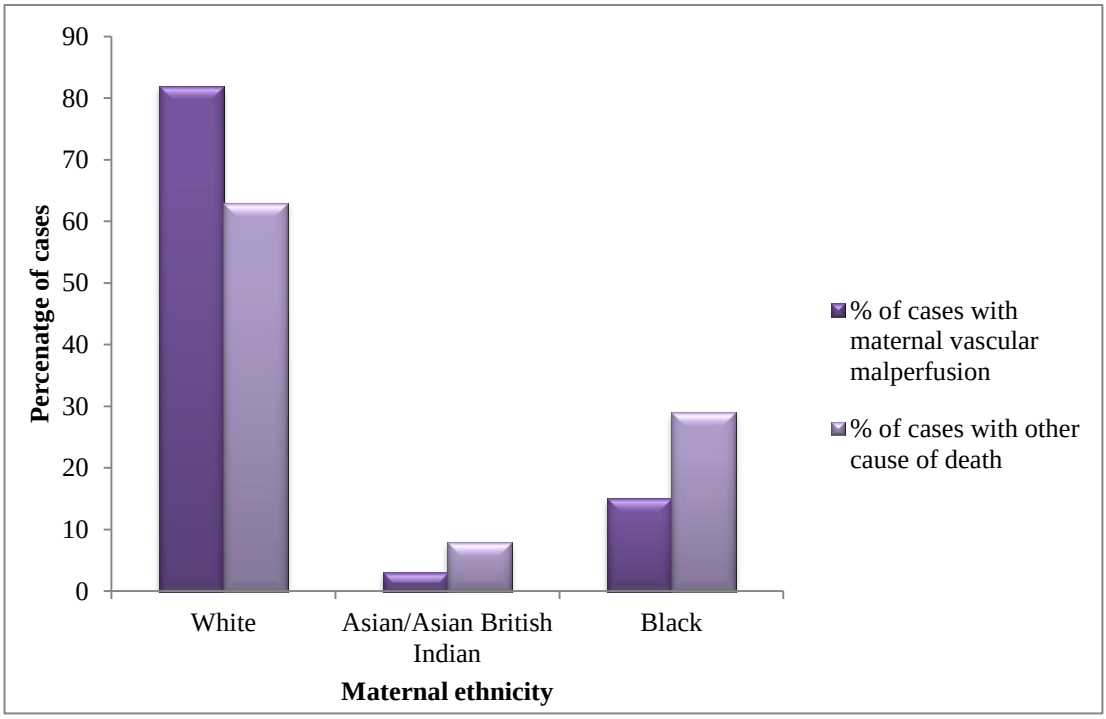


Figure 89 *Maternal ethnicity in cases of maternal vascular malperfusion*

Maternal Age

The majority of mothers were less than or equal to 35 years old in cases of maternal vascular malperfusion and all other causes of death. There was no significant difference in the maternal age distribution between the two groups.

Maternal Age (years)	Number of cases with maternal vascular malperfusion	Number of cases with a different cause of death	**Total**
Less than or equal to 35	32 (78%)	766 (77%)	**798 (77%)**
36-40	4 (10%)	189 (19%)	**193 (19%)**
>40	5 (12%)	43 (4%)	**48 (5%)**
Total:	**41**	**998**	**1039**

Table 112 Maternal Age for cases of maternal vascular malperfusion (excludes 1 case with no maternal age) and cases of all other causes of death.

Figure 90 *Maternal Age for cases of maternal vascular malperfusion and other causes of death*

Maternal BMI

Only 16 cases with maternal vascular malperfusion had a recorded maternal BMI and of those, the majority had a normal BMI. There was no significant difference in the distribution of BMI between cases of maternal vascular malperfusion and all other causes of death.

Maternal BMI	Number of cases with maternal vascular malperfusion	Number of cases with a different cause of death	**Total**
Underweight	0 (0%)	16 (4%)	**16 (3%)**
Normal	7 (44%)	135 (30%)	**142 (30%)**
Overweight	5 (31%)	157 (35%)	**162 (35%)**
Obese	4 (25%)	142 (32%)	**146 (31%)**
Total	**16**	**450**	**466**

Table 113 Maternal BMI for cases of maternal vascular malperfusion

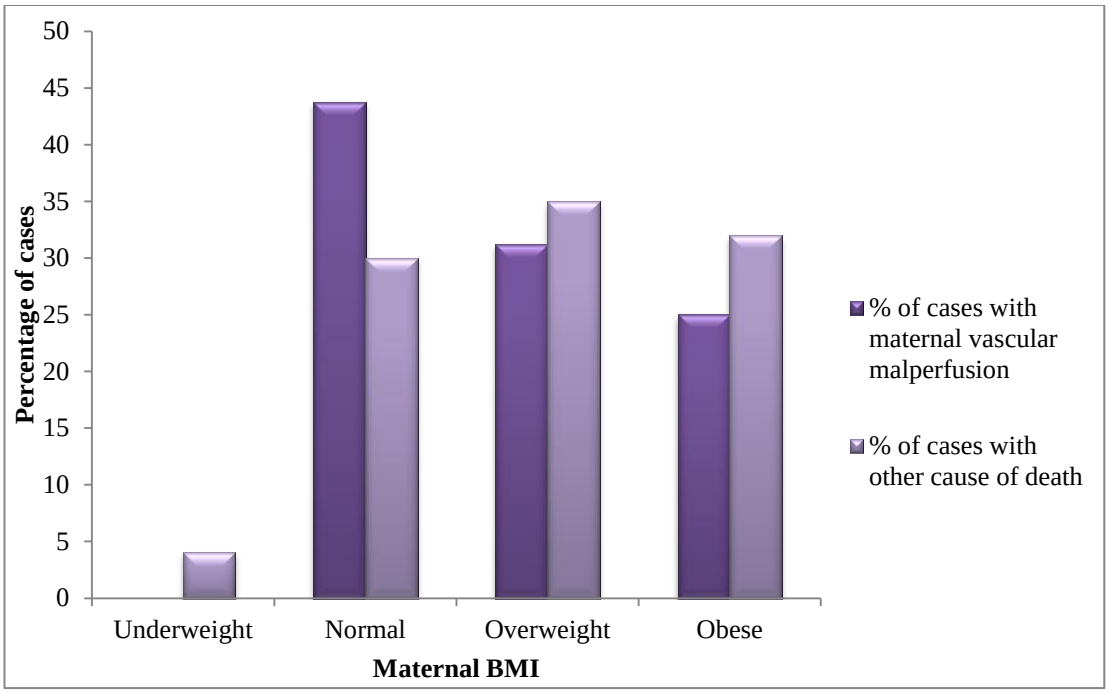


Figure 91 *Maternal BMI for cases of maternal vascular malperfusion and all other causes of death*

Summary

In cases of maternal vascular malperfusion:

- There were two gestational peaks, one at 24 weeks and the other at 28-29 weeks
- The majority of deaths were intrauterine antepartum with >24 hours of intrauterine retention and 98% of all maternal vascular malperfusion cases had some degree of maceration.
- There were significantly more cases of maternal vascular malperfusion in white mothers compared to other ethnicities.
- The majority of mothers with maternal vascular malperfusion were less than or equal to 35 years of age and had normal BMIs
- There was no significant difference in the distribution of maternal age or maternal BMI between cases of maternal vascular malperfusion and other causes of death.

7.2.6 Other specific significant placental pathologies

Maternal vascular malperfusion represented by far the largest proportion of placental abnormalities within the "placenta" cause of death category. However, there were 14 other cases with specific histological abnormalities of the placenta including cases of;

1. Fetal Vascular Occlusion
2. Chronic Histiocytic Intervillositis
3. Massive perivillous fibrin deposition

Table 114 represents the clinical findings in these cases. The majority of the deaths (79%) were intrauterine with a retention time of > 24 hours and fetal maceration varied from mild to severe. The average fetal gestation for these cases was 32 weeks. Two thirds of the mothers were white; mothers had an average age of 31 years and 50% of mothers (3 out of 6) were overweight.

Placental Pathology:	Type of death	Degree of maceration	Fetal gestation	Maternal ethnicity	Maternal BMI	Maternal age
Fetal Vascular Occlusion						
Case 1	Intrauterine death > 24 hours	Moderate	37	White	Not given	18
Case 2	Intrauterine death > 24 hours	Other	41	White	51.1	28
Case 3	Intrauterine death > 24 hours	Mild	25	Black	Not given	29
Case 4	Intrauterine death > 24 hours	Severe	29	White	23.1	40
Case 5	Intrauterine death > 24 hours	Severe	38	White	23.2	30
Chronic Histiocytic intervillositis						
Case 1	Intrauterine death > 24 hours	Other	26	White	24.5	39

Case 2	Intrapartum death	None	24	Not given	Not given	27
Case 3	Intrauterine death > 24 hours	Moderate	29	Asian	Not given	41
Massive perivillous fibrin deposition						
Case 1	Intrauterine death > 24 hours	Mild	35	Not given	Not given	Not given
Case 2	Intrauterine death < 24 hours	None	17	Black	26.2	30
Case 3	Intrauterine death > 24 hours	Severe	39	Asian	28	30
Case 4	Intrauterine death > 24 hours	Severe	35	White	Not given	20
Case 5	Intrauterine death < 24 hours	Mild	27	White	Not given	35
Case 6	Intrauterine death >24 hours	Mild	41	White	Not given	40

Table 114 Case findings in cases of significant other placental pathology

Cases of Chronic Histiocytic Intervillositis all occurred in the late second/early third trimester, whereas cases of Massive perivillous fibrin deposition affected all gestational ages from second trimester to post-term.

7.2.7 Unexplained placental lesions

There were 78 cases (16 early miscarriages, 5 late miscarriages and 57 stillbirths) in which there were histological findings/abnormalities of the placenta that were either not severe enough to be to be the likely cause of death, or whose significance was uncertain but which could not be completely discounted as having contributed to or be associated with death. These cases were labelled “unexplained, lesion placenta” and included abnormalities such as:

- Isolated subjective increased syncytial knots/ accelerated maturation
- Villitis of unknown aetiology (any)
- Scattered fibrin thrombi (Perivillous /subchorionic/ intravascular)

- Focal areas of sclerotic villi with no other features of fetal thrombotic vasculopathy
- Plasma cell deciduitis
- Calcified thrombi in chorionic plate vessels with no downstream effects
- Small peripheral infarcts/ ischaemic changes

The majority of these unexplained deaths with placental lesions (72%) were intrauterine with a retention time of > 24 hours and 66% were severely macerated, very similar to results for totally unexplained unexplained deaths. The gestational age of unexplained placental lesion cases peaked at 35-40+ weeks gestation suggesting such abnormalities are either incidental and are more frequent with increasing gestational age or are associated with late stillbirths. The mothers were mostly between the ages of 26-30 years, were overweight and of white ethnicity. There was no significant difference in either the gestational age for unexplained placental lesion deaths or in the maternal age when comparing to cases of specific placental abnormalities (p=0.64 and p= 0.7884 respectively). However, when comparing to totally unexplained death, unexplained placental lesion deaths were delivered at a significantly more advanced gestation (median 32 weeks versus median 26 weeks) but there was no significant difference in the maternal age distributions between groups (*Table 115*).

Unexplained lesion Placenta:	Unexplained lesion Placenta	Unexplained Unexplained
Type of death		
Intrauterine death < 24 hours	8 (10%)	36 (12%)
Intrauterine death > 24 hours	56 (72%)	196 (67%)
Intrapartum death -Known	2 (3%)	10 (3%)

Intrapartum death - Fresh	12 (15%)	50 (17%)
Degree of maceration		
None	12 (16%)	62 (22%)
Mild	7 (9%)	33 (12%)
Moderate	2 (3%)	20 (7%)
Severe	49 (66%)	120 (42%)
Other	7 (9%)	50 (18%)
Not given	1	7
Fetal gestation		
15-23 weeks	21 (27%)	122 (42%)
24-30 weeks	20 (26%)	49 (17%)
31-34 weeks	5 (6%)	22 (8%)
35- term weeks	32 (41%)	99 (34%)
Maternal ethnicity		
White	40 (69%)	133 (68%)
Black	12 (21%)	45 (23%)
Asian	6 (10%)	11 (6%)
Mixed	0	5 (3%)
Chinese	0	1 (1%)
Not given	20	97
Maternal age		
<20 years	4 (5%)	21 (7%)
21-25 years	10 (13%)	35 (12%)
26-30 years	26 (34%)	67 (24%)

31-35 years	19 (25%)	101 (35%)
35-40 years	15 (20%)	50 (18%)
40+ years	2 (3%)	10 (4%)
Not given	2	8
Maternal BMI		
Underweight	0 (0%)	2 (2%)
Normal	8 (35%)	45 (46%)
Overweight	10 (43%)	47 (48%)
Obese	5 (22%)	3 (3%)
Not given	55	195

Table 115 Case findings in unexplained placental lesions and unexplained unexplained cases

7.3 Discussion

In 946 stillbirth and miscarriage autopsies (89%) the placenta was also submitted for examination. Nearly one third of all placentas had entirely normal microscopic findings of the umbilical cord, membranes and placenta sections. Stillbirths, however, had a third of cases with some type of placental abnormality (a significantly greater proportion than miscarriages) and in 208 of these cases, the placental features could be directly linked to the cause of death, including those with diagnosis based mainly on clinical history, such as abruption. There was a significant difference in the placental weight between black and white mothers, however the placental weight was not adjusted for gestational age and it is known that black mothers had more frequent miscarriages than white mothers (Chapter 3) and this is therefore the likely cause for differences seen. Cord examination found that the majority were eccentrically inserted into the placenta and there was no significant difference in cord insertion between cases of miscarriage and stillbirth. Overall, determination of macroscopic features of the placenta, such as weight and cord

insertion site are of limited value in determining cause of death but macroscopic examination is important for cases of retroplacental haemorrhage/ abruption (in conjunction with the clinical features) and for the identification of focal lesions, such as infarcts, for sampling and subsequent histological examination.

In total, 303 cases in the study population were attributed a cause of death related to placental abnormalities including;

- Placental Abruption
- Ascending Infection
- IUGR
- Specific placental histological abnormalities
- Pre-eclampsia

These deaths account for nearly one third of all causes of death in the study, second only to unexplained deaths (see Chapter 4) in keeping with other studies (151, 152). 58% were ascending infection, almost always associated with miscarriage and intrapartum death, whilst 18% were related to specific histological abnormalities of the placenta; of which maternal vascular malperfusion accounted for the large majority (75%); nearly twice that reported in a previous large American study (215). In view of these findings cases of ascending infection and maternal vascular malperfusion were analysed in greater detail.

Firstly, in cases of ascending infection the majority of deaths were fresh intrapartum deaths with no maceration. In comparison, the majority of maternal vascular malperfusion cases were intrauterine deaths with retention, 98% showing maceration.

The prevalence of these two conditions varies by gestational age; a recognised finding (144, 150, 215). Ascending infection frequency peaks at around 22 weeks, whilst maternal vascular malperfusion is most frequent at 24-29 weeks gestation.

Black mothers were significantly more likely to be affected by cases of ascending infection with an odds ratio of four compared to white mothers. A previous American study reported that intrapartum and early gestational age stillbirths are more common in non-Hispanic black women compared to non-Hispanic white women and Hispanic women, in keeping with the findings of the present study (215). Conversely, white mothers were more likely to be affected by maternal vascular malperfusion than all other ethnicities.

There were no differences in the maternal age or BMI distributions of ascending infection or maternal vascular malperfusion cases versus other causes of death.

Cases with significant other specific placental pathologies causing death (14 of 1064, 1%) were predominantly intrauterine deaths with a retention time of > 24 hours, at an average fetal gestation of 32 weeks, with some degree of maceration present. The findings are similar to those found in cases of maternal vascular malperfusion.

There was an additional group of cases (78 of 1064, 7%) in which the placenta showed some histological abnormality but which was not severe enough to be attributed as the cause of death or was of uncertain significance. The majority of these otherwise unexplained deaths were also intrauterine with a retention time of > 24 hours, with macerated and a similar gestational age, maternal age and ethnic distribution to the overall population, including a predominant occurrence at or near term. Several of these features, such as villitis of unknown aetiology (VUE) and abnormal villous maturation have been reported more frequently in clinically normal

pregnancies delivering at term and therefore have very low positive predictive values (216). At present there is no “gold standard” or objective test to determine whether in an individual case, such findings contributed directly or indirectly to the cause of fetal death and this is an important area of future research.

In conclusion, the findings of this chapter demonstrate that ascending infection is the major cause of late second trimester miscarriage with intrapartum or fresh stillbirth, specially affecting women of black ethnic origin. Other placental histological abnormalities represent a significant cause of death for third trimester stillbirths, especially maternal vascular malperfusion and chronic histiocytic intervillositis in the early third trimester. A range of placental histological abnormalities of uncertain significance remain, particularly in the later third trimester stillbirth, the significance of which is uncertain. Placental examination remains an important contributor to determining cause of death in cases of intrauterine fetal death at all gestational ages, including routine histological sampling and assessment of cord, membranes and placental parenchyma, even when this appears normal macroscopically, since in many cases a specific histological diagnosis to explain the death may be possible.

8. Histological examination

8.0 Background

8.1 Methods

8.2 Results

8.3 Discussion

8.0 Background

It is recommended by The Royal College of Pathologists (UK) that fetal organs should be sampled for histological analysis in stillbirth autopsy but this is based on expert opinion rather than published data (159). Similarly, the Kennedy Guidelines for the investigation of Sudden Unexpected Death in Infancy (SUDI) recommends autopsy procedures and guides pathologists on the tissue that should be taken for histological analysis based on opinion but limited data (217). Recent data suggest much more limited sampling in SUDI would not result in undetected causes of death (218). Stillbirth is 10 times more common than SUDI and yet there are no large scale published studies which evaluate the usefulness and/ or necessity of the histological analysis of specific organs in stillbirth (6).

Although major organ sampling may theoretically lead to detection of specific congenital abnormalities, aiding clinicians in the relevance of these abnormalities for future pregnancies, the most commonly reported causes of death in stillbirth are, IUGR/SGA, ascending infection and 'unexplained' deaths, all of which do not significantly rely on histological analysis of fetal internal organ tissue for their detection other than as an investigation "of exclusion" (5, 175, 194-197).

The aim of this chapter is to therefore evaluate the usefulness of both the macroscopic and microscopic assessment of fetal internal organs. Cases in which the histological analysis of tissue provided the definitive cause of death will be identified.

8.1 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriages from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Data

was analysed through queries and statistical tests run using Microsoft Access, Excel, Graph Pad Prism and Stats Direct. Statistical test results can be viewed in detail in Appendix 3.

8.2 Results

There were 1064 cases within the total study population. Macroscopic and microscopic examinations of organs were completed in the majority of cases (see tables below) and were categorised into four main groups:

1. Normal: no abnormalities found
2. Abnormal BUT not contributed to death: e.g. congestion of tissue
3. Abnormal and potentially contributed to death: e.g. Intraalveolar haemorrhage and aspirated squamous cells within lung tissue
4. Abnormal and definitive cause of death: abnormalities directly provide cause of death e.g. CMV seen on lung histology

Some cases were not consented for macroscopic and / or microscopic examination of organs and were categorised as ‘not examined’. Organs, which in the pathologist’s expert opinion did not require analysis, were also placed in this category. In some instances organs were ‘too autolysed’ for useful examination and thus were categorised as such.

Heart

Heart Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
Normal	234 (95%)	153 (85%)	480 (75%)	**867 (81%)**
Abnormal BUT not contributed to death	4 (2%)	13 (7%)	104 (16%)	**121 (11%)**
Abnormal and potentially contributed to death	2 (1%)	3 (2%)	26 (4%)	**31 (3%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	1 (<1%)	**1 (<1%)**
Not examined	4 (2%)	10 (6%)	28 (4%)	**42 (4%)**
Too autolysed	2 (1%)	0 (0%)	0 (0%)	**2 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 116 Macroscopic heart findings in different death categories

The majority of cases had a normal macroscopic appearance of the heart (81%). Stillbirths had the greatest proportion of macroscopic abnormalities of the heart but almost all were minor changes that did not directly contribute to death (99%). Only one case, a stillborn had a macroscopic cardiac abnormality that provided the definitive cause of death (see histology below).

Heart Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	240 (98%)	160 (89%)	546 (85%)	**946 (89%)**
Abnormal BUT not contributed to death	0 (0%)	5 (3%)	41 (6%)	**46 (4%)**
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	13 (2%)	**13 (1%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	1 (<1%)	**1 (<1%)**
Not examined	4 (2%)	12 (7%)	32 (5%)	**48 (5%)**
Too autolysed	2 (1%)	2 (1%)	5 (1%)	**9 (1%)**
Presumed normal, histo taken, not in PM report	0 (0%)	0 (0%)	1 (<1%)	**1 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 117 Microscopic heart findings in different death categories

The majority of heart histology was normal (89%). Stillbirths had the greatest proportion of microscopic abnormalities of the heart but almost all were minor abnormalities that did not directly contribute to death (98%). Only one heart had a microscopic abnormality that provided a definitive cause of death, which was also seen macroscopically:

1. Epstein anomaly of tricuspid valve enlarged dilated right atrium. The artrialised portion was extremely thin and almost transparent. There was marked displacement of the right atrioventricular valve into the right ventricle.

a. Cause of death given: Congenital abnormalities. (No antenatal details were provided in this case).

Heart	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	234 (100%)	148 (97%)	446 (93%)
Normal macro, Histo abnormal and not COD	0 (0%)	1 (1%)	21 (4%)
Normal macro, Histo possibly related to COD	0 (0%)	0 (0%)	5 (1%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	0 (0%)	2 (1%)	2 (<1%)
Normal macro, Histo too autolysed	0 (0%)	2 (1%)	5 (1%)
Normal macro, Histo presumed normal, not in PM report	0 (0%)	0 (0%)	1 (<1%)
Total:	**234**	**153**	**480**

Table 118 Microscopic histology findings when macroscopic findings normal

The majority of cases (93-100%) had both a normal macroscopic and microscopic examination of the heart. Importantly, there were no cases in this large series of intrauterine deaths in which histological examination provided the cause of death when the macroscopic appearance of the heart was normal.

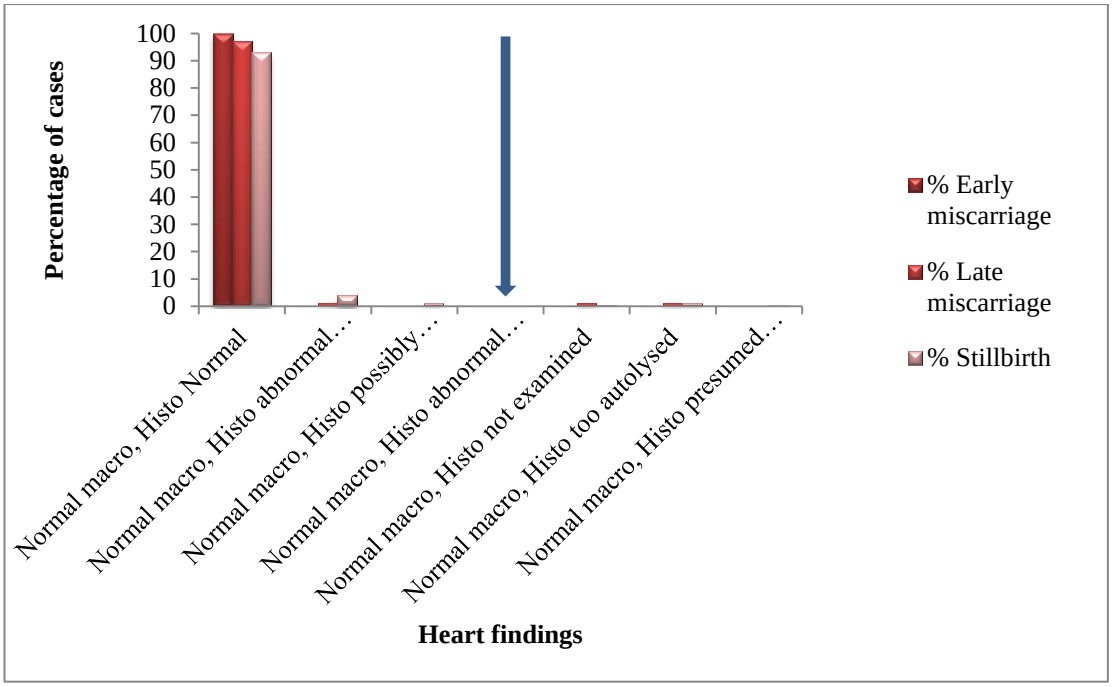


Figure 92 *Microscopic findings in different death categories with normal macroscopic findings in the heart*

Lung

Lung Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	235 (96%)	159 (89%)	448 (70%)	**842 (79%)**
Abnormal BUT not contributed to death	1 (<1%)	6 (3%)	136 (21%)	**143 (13%)**
Abnormal and potentially contributed to death	4 (2%)	3 (2%)	25 (4%)	**32 (3%)**
Abnormal and definitive cause of death	0 (0%)	1 (1%)	2 (<1%)	**3 (<1%)**
Not examined	4 (2%)	10 (6%)	28 (4%)	**42 (4%)**
Too autolysed	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Not given	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 119 Macroscopic findings in the lung in different death categories

The majority of cases had a normal macroscopic appearance of the lungs (79%). Stillbirths had the greatest proportion of macroscopic abnormalities of the lungs but almost all were minor changes that did not directly contribute to death (99%). In only three cases did the lungs have a macroscopic abnormality that provided a definitive cause of death (see histology below).

Lung Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
Normal	198 (80%)	100 (56%)	198 (31%)	**496 (47%)**
Abnormal BUT not contributed to death	25 (10%)	24 (13%)	323 (51%)	**372 (35%)**
Abnormal and potentially contributed to death	14 (6%)	32 (18%)	72 (11%)	**118 (11%)**
Abnormal and definitive cause of death	2 (1%)	8 (4%)	11 (2%)	**21 (2%)**
Not examined	5 (2%)	11 (6%)	32 (5%)	**48 (5%)**
Too autolysed	2 (1%)	4 (2%)	3 (<1%)	**9 (1%)**
Total:	**246**	**179**	**639**	**1064**

Table 120 Microscopic histological findings in different causes of death

The majority of lung histology was normal (47%). Stillbirths had the greatest proportion of microscopic abnormalities of the lungs but almost all were minor abnormalities that did not directly contribute to death (97%). In 21 cases there were microscopic abnormalities of the lungs that provided a definitive cause of death including:

- Eighteen cases of pneumonia indicating ascending infection
    - 14 had placentas submitted; all showed chorioamnionitis in the placenta and/or funisitis/angiitis of the cord.
    - 1 case had no placenta submitted but showed necrotizing funisitis of the cord
- Two cases of Cytomegalovirus infection (CMV)
    - One case had no placenta submitted for examination

    - One case showed evidence of choirioamnionitis but no CMV inclusions within the placenta
- One case of congenital pulmonary airway malformation
    - The placental examination showed acute atherosis

Twenty of the 21 cases had causes of death secondary to infection, which theoretically could have been identified on placental examination alone. 16 of the 21 cases (76%) had placentas submitted for histological examination. Placental examination showed evidence of chorioamnionitis and/ or funisitis within the cord in all cases of ascedning infection in which a placenta was submitted (14 cases). One case of ascendign infection showed necrotising funisitis within the cord despite no placenta being submitted for examination.

Of the two cases diagnosed with CMV, one case had no placenta submitted and the other showed chorioamnionitis but no CMV inclusions in the placenta.

Placental examination in the case of congenital pulmonary airway malformation showed acute atherosis, hence the cause of the intrauterine death could have been primary placental disease rather than pulmonary related.

These findings show that 15 of the 21 cases (71%) could have been diagnosed through placental examination alone. The findings also suggest that the if the other three cases of ascending infection had placentas submitted for examination, it would be very likely that they would show positive finindgs for chorioamnionitis, meaning up to 18 of the 21 cases (86%) could be diagnosed through placental examination only.

Lungs	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	196 (83%)	96 (60%)	172 (38%)
Normal macro, Histo abnormal and not COD	25 (11%)	23 (14%)	216 (48%)
Normal macro, Histo possibly related to COD	13 (6%)	28 (18%)	50 (11%)
Normal macro, Histo abnormal and COD	2 (1%)	7 (4%)	5 (1%)
Normal macro, Histo not examined	0 (0%)	2 (1%)	2 (<1%)
Normal macro, Histo too autolysed	0 (0%)	3 (2%)	3 (1%)
Total:	**236**	**159**	**448**

Table 121 Microscopic findings when macroscopic findings are normal in different cases of death

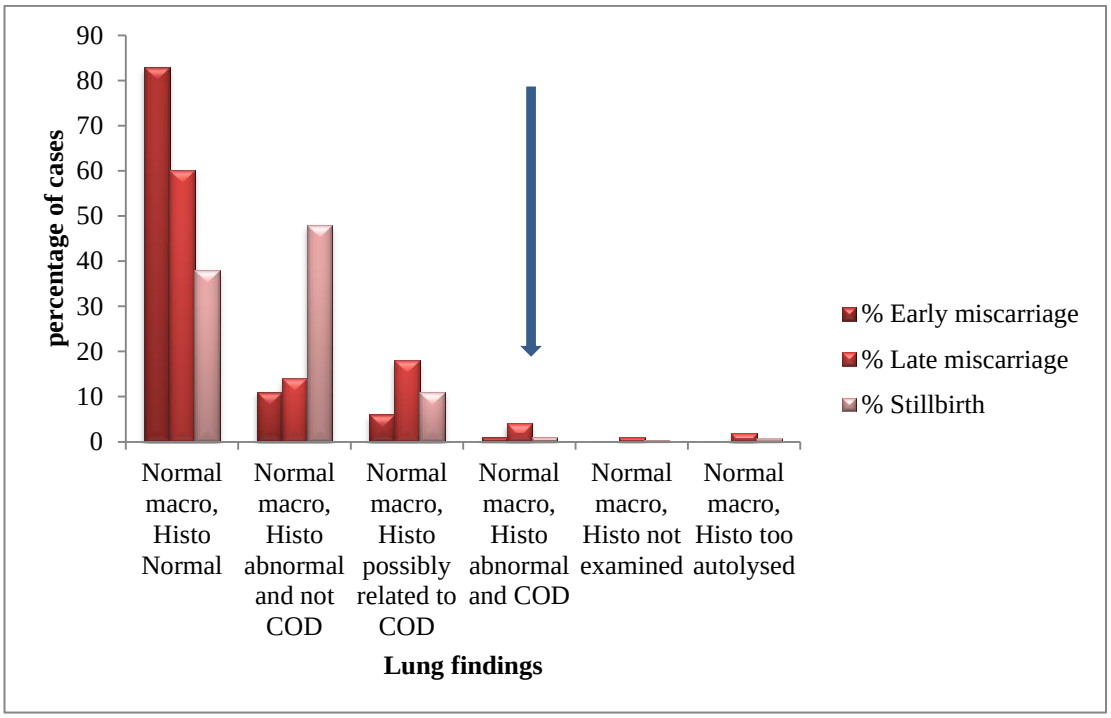


Figure 93 *Microscopic findings in different death categories with normal macroscopic findings in the Lung*

The majority of cases had both a normal macroscopic and microsocpic appearance of the lungs. In 14 cases (2%) the macroscopic appearance of the lungs was normal but the histology was abnormal; 14 cases showed pneumonia and 1 case had CMV infection. The histology therefore gave a definitive cause of death. Howerer, 10 of

these cases had placentas submitted for histological examination; 9 cases showed evidence of chorioamnionitis within the placenta. The remianing case, despite showing chorioamnionitis, did not show CMV inclusions which was deemed the cause of death in that case.

Liver

Liver Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
Normal	226 (92%)	159 (89%)	530 (83%)	**915 (86%)**
Abnormal BUT not contributed to death	10 (4%)	8 (4%)	70 (11%)	**88 (8%)**
Abnormal and potentially contributed to death	1 (<1%)	1 (1%)	7 (1%)	**9 (1%)**
Abnormal and definitive cause of death	0 (0%)	1 (1%)	1 (<1%)	**2 (<1%)**
Not examined	4 (2%)	10 (6%)	29 (5%)	**43 (4%)**
Too autolysed	4 (2%)	0 (0%)	2 (<1%)	**6 (1%)**
Not given	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 122 Macroscopic liver findings in different death categories

The majority of cases had a normal macroscopic appearance of the liver (86%). Stillbirths had the greatest proportion of macroscopic abnormalities of the liver but almost all were minor changes that did not directly contribute to death (99%). In only two cases was the liver was found to have macroscopic abnormalities that provided a definitive cause of death (see histology below).

Liver Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
Normal	226 (92%)	151 (84%)	494 (77%)	**871 (82%)**
Abnormal BUT not contributed to death	6 (2%)	13 (7%)	87 (14%)	**106 (10%)**
Abnormal and potentially contributed to death	2 (1%)	1 (1%)	9 (1%)	**12 (1%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	1 (<1%)	**1 (<1%)**
Not examined	6 (2%)	12 (7%)	35 (3%)	**53 (5%)**
Too autolysed	6 (2%)	2 (1%)	13 (2%)	**21 (2%)**
Total:	**246**	**179**	**639**	**1064**

Table 123 Microscopic Liver findings in different death categories

The majority of liver histology was normal (82%). Stillbirths had the greatest proportion of microscopic abnormalities of the liver but almost all were minor abnormalities that did not directly contribute to death (99%). There was only one case in which microscopic examination of the liver provided the definitive cause of death, however in this case the diagnosis would have been made by examination of the lung and/or the brain.

1. Numerous abscesses with multiple gram positive bacilli
    a. Cause of death given: Infection

Liver	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	217 (96%)	144 (91%)	448 (85%)
Normal macro, Histo abnormal and not COD	5 (2%)	11 (7%)	61 (12%)
Normal macro, Histo possibly related to COD	0 (0%)	0 (0%)	5 (1%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	1 (<1%)	2 (1%)	5 (1%)
Normal macro, Histo too autolysed	3 (1%)	2 (1%)	11 (2%)
Total:	**226**	**159**	**530**

Table 124 Microscopic liver findings when macroscopic findings normal in different causes of death

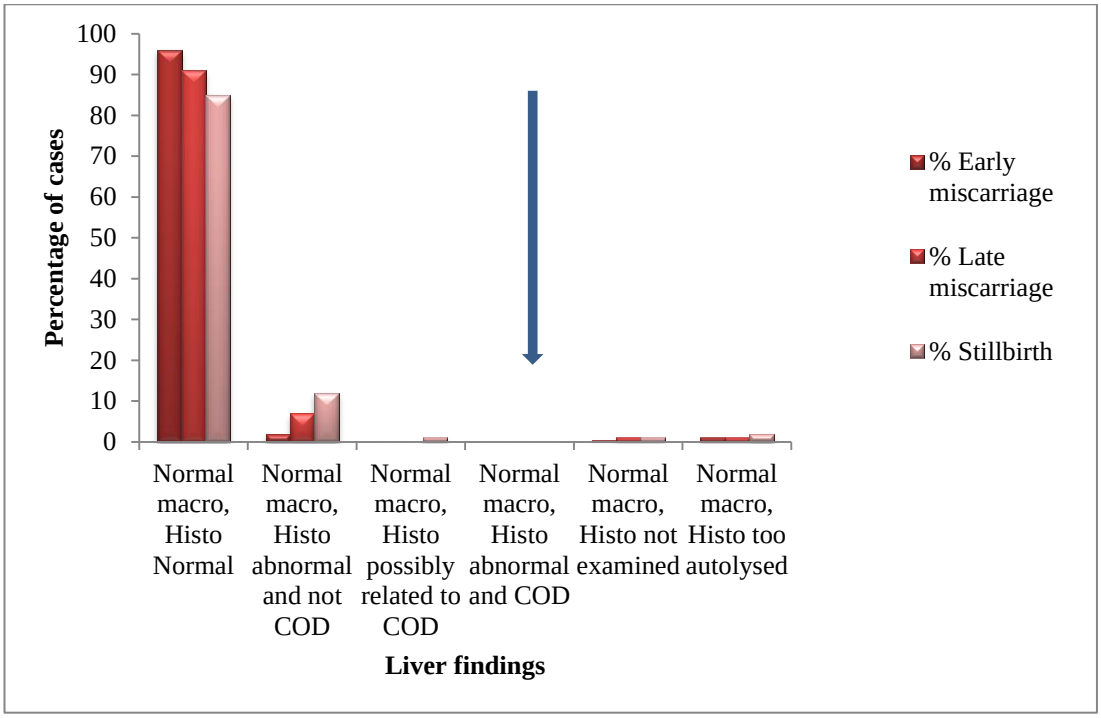


Figure 94 *Microscopic findings in different death categories with normal macroscopic findings in the Liver*

The majority of all cases had both a normal macroscopic and microscopic appearance of the liver. There were no cases with a normal macroscopic appearance with abnormal histology that was a definite cause of death.

Kidney

Kidney Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
Normal	231 (94%)	164 (92%)	484 (76%)	**879 (83%)**
Abnormal BUT not contributed to death	3 (1%)	3 (2%)	114 (18%)	**120 (11%)**
Abnormal and potentially contributed to death	5 (2%)	2 (1%)	8 (1%)	**15 (1%)**
Abnormal and definitive cause of death	1 (<1%)	0 (0%)	2 (<1%)	**3 (<1%)**
Not examined	4 (2%)	10 (6%)	30 (5%)	**44 (4%)**
Too autolysed	2 (1%)	0 (0%)	1 (<1%)	**3 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 125 Macroscopic findings in the kidney for different death categories

The majority of cases had a normal macroscopic appearance of the kidneys (83%). Stillbirths had the greatest proportion of macroscopic abnormalities of the kidneys but almost all were minor changes that did not directly contribute to death (95%). In only 3 cases the kidney was found to have macroscopic abnormalities that provided a definitive cause of death, including one case of bilateral renal agenesis (see histology below).

Kidney Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total:**
Normal	228 (93%)	132 (74%)	487 (76%)	**847 (80%)**
Abnormal BUT not contributed to death	8 (3%)	26 (15%)	98 (15%)	**132 (12%)**
Abnormal and potentially contributed to death	2 (1%)	6 (3%)	8 (1%)	**16 (2%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	5 (1%)	**5 (<1%)**
Not examined	6 (2%)	13 (7%)	36 (6%)	**55 (5%)**
Too autolysed	2 (1%)	2 (1%)	5 (1%)	**9 (1%)**
Total:	**246**	**179**	**639**	**1064**

Table 126 Microscopic findings in the kidney for different death categories

The majority of kidney histology was normal (80%). Stillbirths had the greatest proportion of microscopic abnormalities of the kidneys but almost all were minor abnormalities that did not directly contribute to death (95%). There were five cases in which a microscopic abnormality in the kidney provided the definitive cause of death:

1. Absent kidneys with only a small amount of renal tissue on the left.
    a. Cause of death given: Congenital abnormalities (mother had no antenatal care; no history of scans)

2. Large mass, up to 7cm diameter, right kidney, consistent with mesoblastic nephroma.

a. Cause of death given: Congenital abnormalities (seen on antenatal scan)

3. The right kidney showed patchy, mainly non-occlusive focally calcified laminated thrombus within the renal vein and its teributary intrarenal branches, suggestive of a fetal thrombotoic tendancy (fetal thrombotic vasculopathy FTV).
    a. Cause of death given: Placenta

4. Right kidney shows a focally calcified laminated thrombus in the renal vein and its tributary intrarenal branches, indicating the likely presence of a fetal thrombophilia.
    a. Cause of death given: Placenta

5. Scattered cytomegalic cells containing nuclear inclusions
    a. Cause of death given: Infection (also detected on Lung histology)

Kidney	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	221 (96%)	129 (79%)	401 (83%)
Normal macro, Histo abnormal and not COD	8 (3%)	24 (15%)	68 (14%)
Normal macro, Histo possibly related to COD	0 (0%)	6 (4%)	4 (1%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	2 (<1%)
Normal macro, Histo not examined	1 (<1%)	3 (2%)	5 (1%)
Normal macro, Histo too autolysed	1 (<1%)	2 (1%)	4 (1%)
Total:	**231**	**164**	**484**

Table 127 Microscopic findings in the kidney with normal macroscopic findings for different death categories

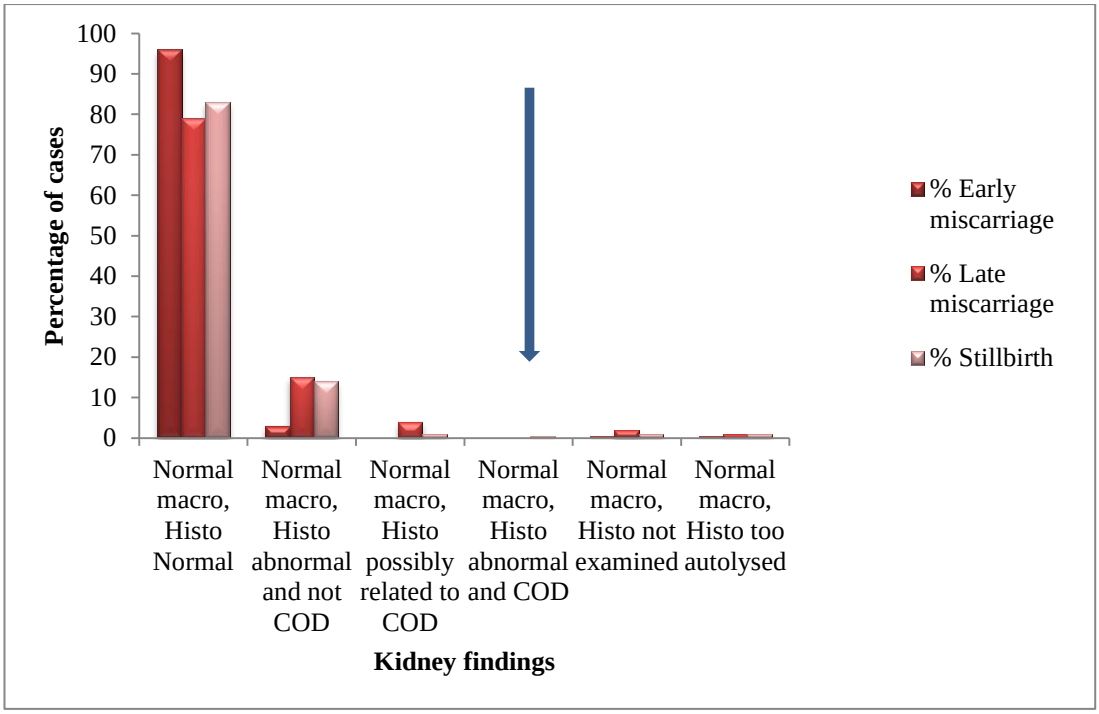


Figure 95 *Microscopic findings in different death categories with normal macroscopic findings in the kidney*

The majority of cases (79-96%) had both a normal macroscopic and microscopic appearance of the kidneys. Only two cases had a normal macroscopic appearance and an abnormal histological appearance which was directly linked to the cause of death. On both occasions the cause of death was linked to fetal thrombosis. However, both cases showed extensive placental FTV and the underlying diagnosis would have been identified without fetal histological sampling.

Brain

Brain Macro	Early Miscarriage	Late miscarriage	Stillbirth	Total:
Normal	185 (75%)	124 (69%)	339 (53%)	648 (61%)
Abnormal BUT not contributed to death	16 (7%)	25 (14%)	200 (31%)	241 (23%)
Abnormal and potentially contributed to death	5 (2%)	6 (3%)	35 (5%)	46 (4%)
Abnormal and definitive cause of death	0 (0%)	0 (0%)	6 (1%)	6 (1%)
Not examined	15 (6%)	13 (7%)	49 (8%)	77 (7%)
Too autolysed	25 (10%)	11 (6%)	10 (2%)	46 (4%)
Total:	**246**	**179**	**639**	**1064**

Table 128 Macroscopic findings in the brain for different death categories

The majority of cases had a normal macroscopic appearance of the brain (61%). Stillbirths had the greatest proportion of macroscopic abnormalities of the brain but almost all were minor changes that did not directly contribute to death (98%). In six cases the brain was found to have macroscopic abnormalities that provided a definitive cause of death (see histology below).

Brain Histology	Early Miscarriage	Late miscarriage	Stillbirth	Total:
Normal	184 (75%)	126 (70%)	298 (47%)	608 (57%)
Abnormal BUT not contributed to death	10 (4%)	23 (13%)	206 (32%)	239 (22%)
Abnormal and potentially contributed to death	3 (1%)	7 (4%)	52 (8%)	62 (6%)
Abnormal and definitive cause of death	0 (0%)	0 (0%)	3 (1%)	3 (<1%)
Not examined	22 (9%)	16 (9%)	61 (10%)	99 (9%)
Too autolysed	26 (11%)	7 (4%)	16 (3%)	49 (5%)
Not given	1 (<1%)	0 (0%)	0 (0%)	1 (<1%)
Pres norm, "PM histo" taken but organ NOT reported	0 (0%)	0 (0%)	2 (<1%)	2 (<1%)
No information provided	0 (0%)	0 (0%)	1 (<1%)	1 (<1%)
Total:	**246**	**179**	**639**	**1064**

Table 129 Microscopic findings in the brain for different death categories

The majority of brain histology was normal (57%). Stillbirths had the greatest proportion of microscopic abnormalities of the brain but almost all were minor abnormalities that did not directly contribute to death (99%). There were three cases in which histological examination of the brain provided a definitive cause of death:

1. Massive subdural and subarachnoid haemorrhage. Some focal white matter cortical haemorrhage in cerebellum.
    a. Cause of death given: Congenital abnormalities

2. Massive fresh haemorrhage including the germinal matrix and periventricular white matter with microscopic fresh haemorrhage in the white matter.
    a. Cause of death given: Congenital abnormalities

3. Fibrinopurulent leptomeningitis and purulent ventriculitis. Gram positive organisms present. (abnormalities of infection also seen on histology of lung and liver)
    a. Cause of death given: Infection

The intracerebral bleeds could have been visualised on a postmortem MRI and the case of infection showed evidence of infection within the lung and liver tissue also.

Brain	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	168 (91%)	107 (86%)	220 (65%)
Normal macro, Histo abnormal and not COD	4 (2%)	11 (9%)	82 (24%)
Normal macro, Histo possibly related to COD	0 (0%)	2 (2%)	17 (5%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	2 (1%)	3 (2%)	9 (3%)
Normal macro, Histo too autolysed	10 (5%)	1 (1%)	9 (3%)
Not given	1 (1%)	0 (0%)	0 (0%)
Total:	**185**	**124**	**337**

Table 130 Microscopic findings in the Brain with normal macroscopic findings for different death categories (excludes 2 cases with inadequate documentation)

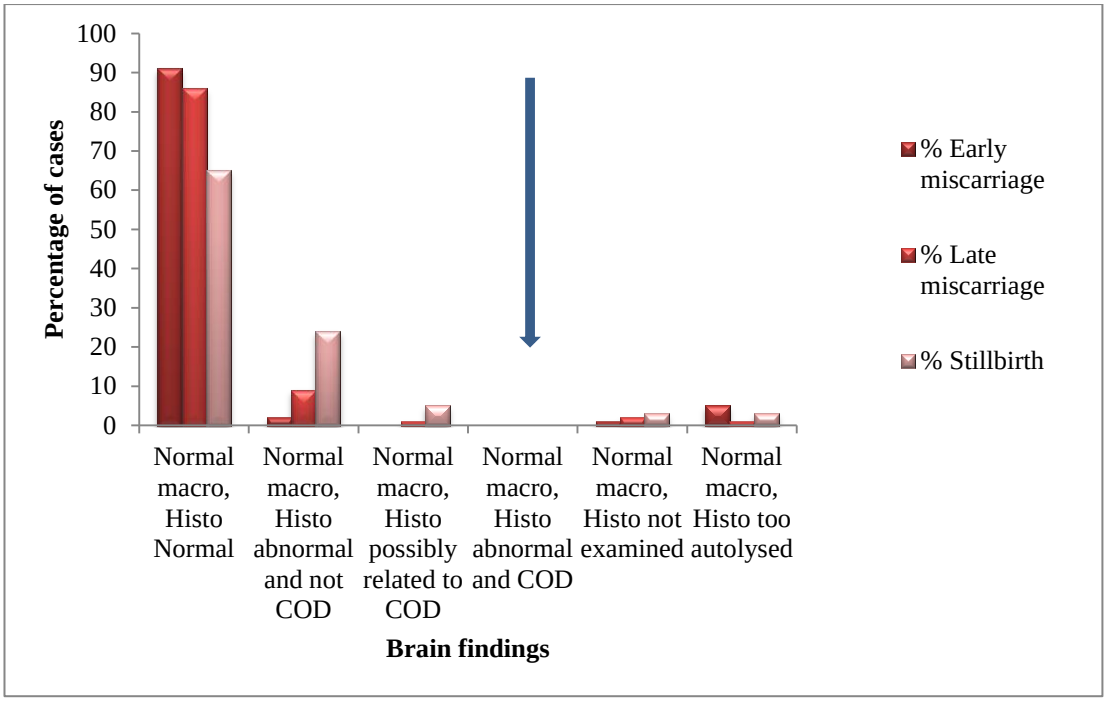


Figure 96 *Microscopic findings in different death categories with normal macroscopic findings in the Brain*

The majority of cases had both a normal macroscopic and microscopic appearance of the brain. There were no cases in which the macroscopic appearance was normal with the microscopic examination providing the definitive cause of death.

Adrenal Glands

Adrenal Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	239 (97%)	166 (93%)	533 (83%)	**938 (88%)**
Abnormal BUT not contributed to death	0 (0%)	2 (1%)	70 (11%)	**72 (7%)**
Abnormal and potentially contributed to death	1 (<1%)	1 (1%)	4 (1%)	**6 (1%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	4 (2%)	10 (6%)	30 (5%)	**44 (4%)**
Too autolysed	2 (1%)	0 (0%)	2 (<1%)	**4(<1%)**
Not given	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Total:	**246**	**179**	**639**	**1064**

Table 131 Macroscopic findings in the adrenal glands for different death categories

The majority of cases had a normal macroscopic appearance of the adrenal glands (88%). Stillbirths had the greatest proportion of macroscopic abnormalities of the adrenal glands and all were minor changes that did not directly contribute to death. There were no cases in which abnormal macroscopic abnormalities of the adrenal glands provided a definitive cause of death.

Adrenal Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	227 (92%)	143 (80%)	422 (66%)	**792 (74%)**
Abnormal BUT not contributed to death	8 (3%)	18 (10%)	153 (24%)	**179 (17%)**
Abnormal and potentially contributed to death	2 (1%)	3 (2%)	17 (3%)	**22 (2%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	7 (3%)	12 (7%)	38 (6%)	**57 (5%)**
Too autolysed	2 (1%)	3 (2%)	9 (1%)	**14 (1%)**
Not given	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Total:	**246**	**179**	**639**	**1064**

Table 132 Microscopic findings in the adrenal glands for different death categories

The majority of adrenal histology was normal (74%). Stillbirths had the greatest proportion of microscopic abnormalities of the adrenal glands and all were minor abnormalities that did not directly contribute to death. There were no cases in which a microscopic abnormality in the adrenal gland provided the definitive cause of death:

Adrenal	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	226 (95%)	141 (85%)	396 (74%)
Normal macro, Histo abnormal and not COD	8 (3%)	17 (10%)	111 (21%)
Normal macro, Histo possibly related to COD	1 (<1%)	3 (2%)	13 (2%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	3 (1%)	2 (1%)	6 (1%)
Normal macro, Histo too autolysed	1 (<1%)	3 (2%)	7 (1%)
Total:	**239**	**166**	**533**

Table 133 Microscopic findings in the adrenal glands with normal macroscopic findings for different death categories

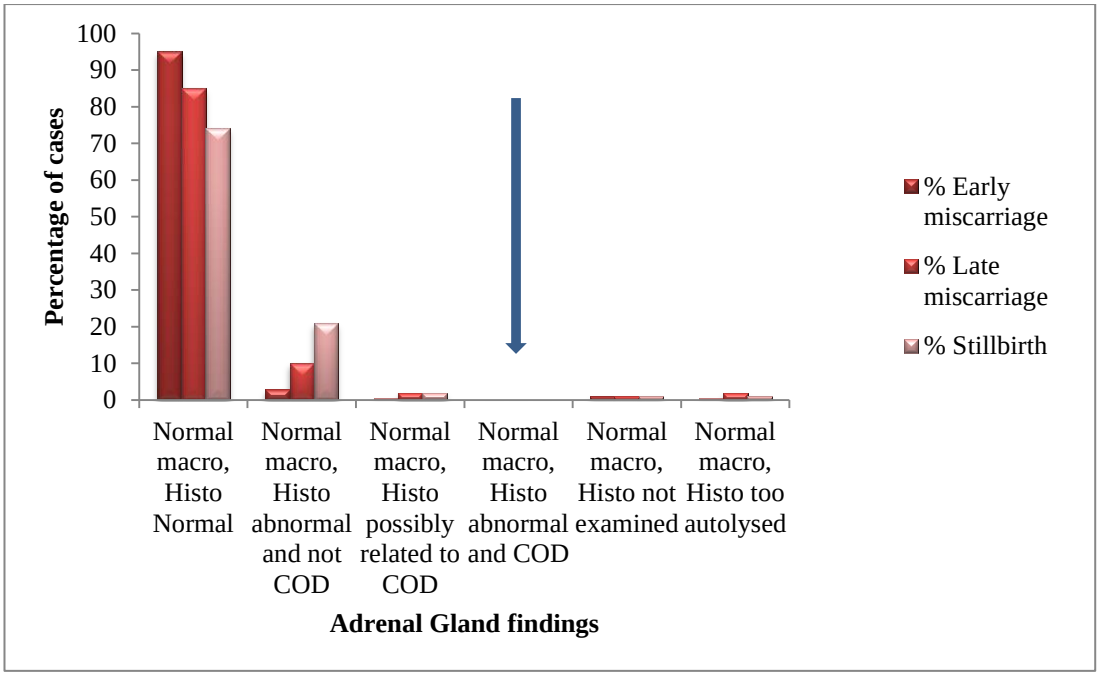


Figure 97 *Microscopic findings in different death categories with normal macroscopic findings in the adrenal glands*

The majority of cases had both a normal macroscopic and microscopic appearance of the adrenal glands. There were no cases in which the macroscopic appearance was normal with the microscopic examination providing the definitive cause of death.

Spleen

Spleen Macro	Early Miscarriage	Late miscarriage	Stillbirth	Total
Normal	219 (89%)	168 (94%)	584 (91%)	971 (91%)
Abnormal BUT not contributed to death	4 (2%)	1 (1%)	23 (4%)	28 (3%)
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Not examined	7 (3%)	10 (6%)	30 (5%)	47 (4%)
Too autolysed	15 (6%)	0 (0%)	2 (<1%)	17 (2%)
Not given	1 (<1%)	0 (0%)	0 (0%)	1 (<1%)
Total:	**246**	**179**	**639**	**1064**

Table 134 Macroscopic findings in the spleen for different death categories

The majority of cases had a normal macroscopic appearance of the spleen (91%). Stillbirths had the greatest proportion of macroscopic abnormalities of the spleen and all were minor changes that did not directly contribute to death. There were no cases in which abnormal macroscopic abnormalities of the spleen provided a definitive cause of death.

Spleen Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	219 (89%)	158 (88%)	512 (80%)	**889 (84%)**
Abnormal BUT not contributed to death	3 (1%)	4 (2%)	50 (8%)	**57 (5%)**
Abnormal and potentially contributed to death	1 (<1%)	0 (0%)	3 (<1%)	**4 (<1%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	13 (5%)	14 (8%)	37 (6%)	**64 (6%)**
Too autolysed	10 (4%)	3 (2%)	37 (6%)	**50 (5%)**
Not given	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Total:	**246**	**179**	**639**	**1064**

Table 135 Microscopic findings in the spleen for different death categories

The majority of spleen histology was normal (84%). Stillbirths had the greatest proportion of microscopic abnormalities of the spleen and all were minor abnormalities that did not directly contribute to death. There were no cases in which a microscopic abnormality in the spleen provided the definitive cause of death.

Spleen	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	211 (96%)	160 (95%)	497 (85%)
Normal macro, Histo abnormal and not COD	3 (1%)	3 (2%)	43 (7%)
Normal macro, Histo possibly related to COD	1 (<1%)	0 (0%)	3 (1%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	3 (1%)	3 (0%)	7 (1%)
Normal macro, Histo too autolysed	1 (<1%)	2 (1%)	34 (6%)
Not given	1 (<1%)	0 (0%)	0 (0%)
Total:	**219**	**168**	**584**

Table 136 Microscopic findings in the spleen with normal macroscopic findings for different death categories

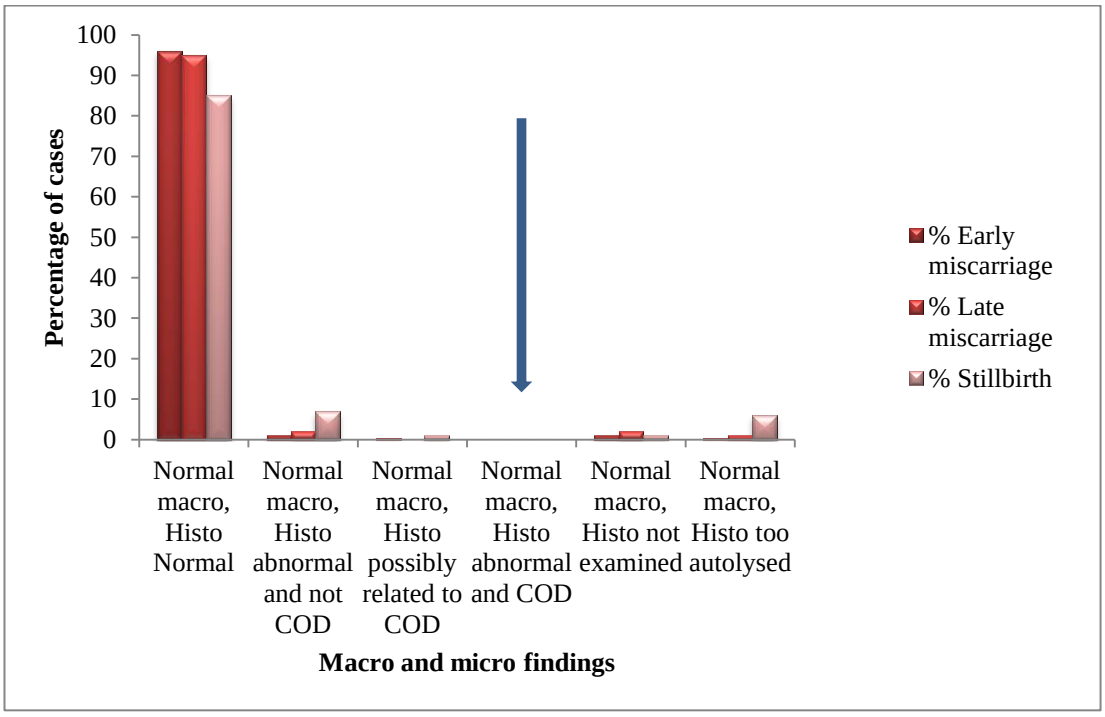


Figure 98 *Microscopic findings in different death categories with normal macroscopic findings in the spleen*

The majority of cases had both a normal macroscopic and microscopic appearance of the spleen. There were no cases in which the macroscopic appearance was normal with the microscopic examination providing the definitive cause of death.

Thymus

Thymus Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	235 (96%)	169 (94%)	550 (86%)	**954 (90%)**
Abnormal BUT not contributed to death	1 (<1%)	0 (0%)	58 (9%)	**59 (6%)**
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	1 (<1%)	**1 (<1%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	5 (2%)	10 (6%)	30 (5%)	**45 (4%)**
Too autolysed	4 (2%)	0 (0%)	0 (0%)	**4 (<1%)**
Not given	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Total:	**246**	**179**	**639**	**1064**

Table 137 Macroscopic findings in the thymus for different death categories

The majority of cases had a normal macroscopic appearance of the thymus (90%). Stillbirths had the greatest proportion of macroscopic abnormalities of the thymus and all were minor changes that did not directly contribute to death. There were no cases in which abnormal macroscopic abnormalities of the thymus provided a definitive cause of death.

Thymus Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	203 (83%)	116 (65%)	409 (64%)	**730 (69%)**
Abnormal BUT not contributed to death	28 (11%)	42 (23%)	173 (27%)	**243 (23%)**
Abnormal and potentially contributed to death	4 (2%)	7 (4%)	18 (3%)	**29 (3%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	9 (4%)	12 (7%)	35 (5%)	**56 (5%)**
Too autolysed	2 (1%)	2 (1%)	4 (1%)	**8 (1%)**
Not given	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Total:	**246**	**179**	**639**	**1064**

Table 138 Microscopic findings in the thymus for different death categories

The majority of thymus gland histology was normal (69%). Stillbirths had the greatest proportion of microscopic abnormalities of the thymus gland and all were minor abnormalities that did not directly contribute to death. There were no cases in which a microscopic abnormality in the thymus gland provided the definitive cause of death.

Thymus	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	201 (86%)	116 (69%)	390 (71%)
Normal macro, Histo abnormal and not COD	28 (12%)	42 (25%)	134 (24%)
Normal macro, Histo possibly related to COD	4 (2%)	7 (4%)	17 (3%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	2 (1%)	2 (1%)	5 (1%)
Normal macro, Histo too autolysed	0 (0%)	2 (1%)	4 (1%)
Total:	**235**	**169**	**550**

Table 139 Microscopic findings in the thymus with normal macroscopic findings for different death categories

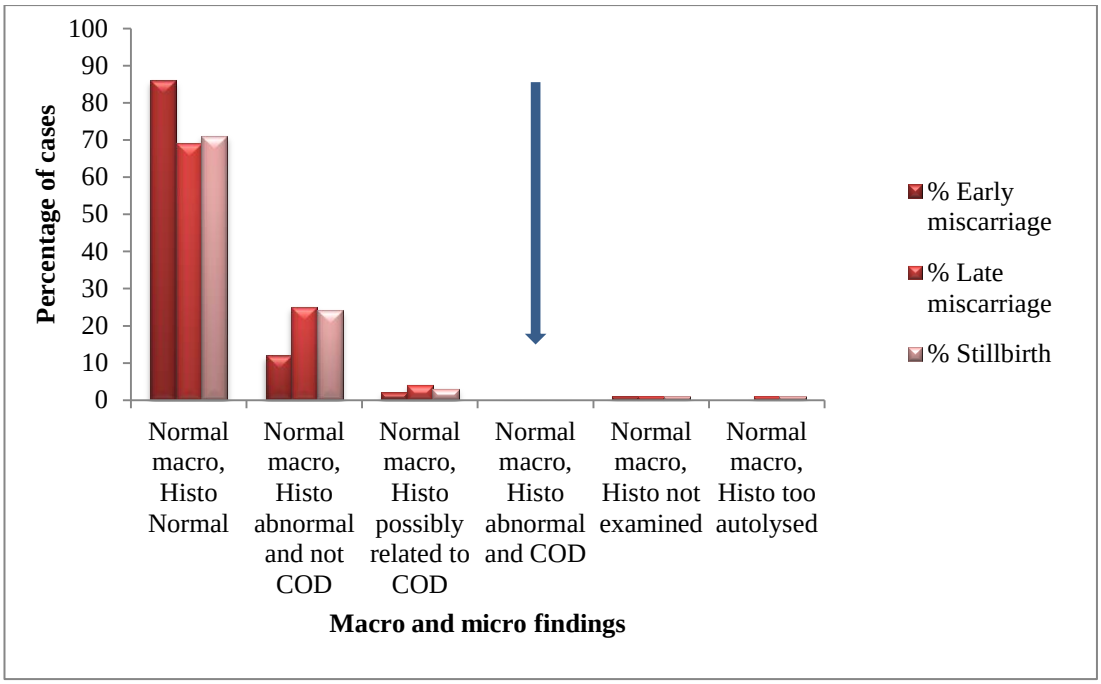


Figure 99 *Microscopic findings in different death categories with normal macroscopic findings in the thymus*

The majority of cases had both a normal macroscopic and microscopic appearance of the thymus. There were no cases in which the macroscopic appearance was normal with the microscopic examination providing the definitive cause of death.

Intestines

Intestine Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	230 (93%)	163 (91%)	581 (91%)	**974 (92%)**
Abnormal BUT not contributed to death	6 (2%)	1 (1%)	10 (2%)	**17 (2%)**
Abnormal and potentially contributed to death	4 (2%)	3 (2%)	13 (2%)	**20 (2%)**
Abnormal and definitive cause of death	0 (0%)	1 (1%)	2 (<1%)	**3 (<1%)**
Not examined	4 (2%)	10 (6%)	29 (5%)	**43 (4%)**
Too autolysed	2 (1%)	1 (1%)	4 (1%)	**7 (1%)**
Not given	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Total:	**246**	**179**	**639**	**1064**

Table 140 Macroscopic findings in the intestines for different death categories

The majority of cases had a normal macroscopic appearance of the intestines (92%). Stillbirths had the greatest proportion of macroscopic abnormalities of the intestines but almost all were minor changes that did not directly contribute to death (82%). There were 3 cases in which abnormal macroscopic abnormalities of the intestines provided a definitive cause of death:

1. Intestinal malrotation; the large and small intestine, together with the mesentery had herniated through a traumatic defect in the left flank beneath the left costal margin..
    a. Cause of death: Congenital abnormalities (no abnormalities noted in antenatal history)
2. Meconium peritonitis secondary to (spontaneous) perforation of the small bowel.
    a. Cause of death: Unexplained lesion, baby (no evidence of abnormalities in antenatal history)

3. Patent urachus and small intestine is present attached to the umbilicus and malrotation with a small mesentery and a huge blind ending rectum is present in the upper pelvis.
    a. Cause of death: Congenital abnormalities (mother had no antenatal care)

Intestinal histology was not taken in the majority of cases (67%).

Intestine Histology	Early Miscarriage	Late miscarriage	Stillbirth
Yes	46 (19%)	27 (15%)	278 (44%)
No	200 (81%)	152 (85%)	361 (56%)
Total:	**246**	**179**	**639**

Table 141 The number and percentage of cases in which intestines examined microscopically

Pancreas

Pancreas Macro	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	225 (91%)	163 (91%)	587 (92%)	**975 (92%)**
Abnormal BUT not contributed to death	0 (0%)	0 (0%)	10 (2%)	**10 (1%)**
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	9 (4%)	15 (8%)	33 (5%)	**57 (5%)**
Too autolysed	10 (4%)	1 (1%)	5 (1%)	**16 (2%)**
Not given	2 (1%)	0 (0%)	4 (1%)	**6 (1%)**
Total:	246	179	639	**1064**

Table 142 Macroscopic findings in the pancreas for different death categories

The majority of cases had a normal macroscopic appearance of the pancreas (92%). Stillbirths had the greatest proportion of macroscopic abnormalities of the pancreas and all were minor changes that did not directly contribute to death. There were no cases in which abnormal macroscopic abnormalities of the pancreas provided a definitive cause of death.

Pancreas Histology	Early Miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	219 (89%)	152 (85%)	423 (66%)	**794 (75%)**
Abnormal BUT not contributed to death	1 (<1%)	8 (4%)	8 (1%)	**17 (2%)**
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	2 (<1%)	**2 (<1%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	16 (7%)	18 (10%)	47 (7%)	**81 (8%)**
Too autolysed	10 (4%)	1 (1%)	159 (25%)	**170 (16%)**
Total:	**246**	**179**	**639**	**1064**

Table 143 Microscopic findings in the pancreas for different death categories

The majority of pancreas histology was normal (79%). Stillbirths had the greatest proportion of microscopic abnormalities of the pancreas and all were minor abnormalities that did not directly contribute to death. There were no cases in which a microscopic abnormality in the pancreas provided the definitive cause of death.

Pancreas	Early miscarriage	Late miscarriage	Stillbirth
Normal macro, Histo Normal	219 (97%)	135 (83%)	415 (71%)
Normal macro, Histo abnormal and not COD	1 (<1%)	7 (4%)	7 (1%)
Normal macro, Histo possibly related to COD	0 (0%)	0 (0%)	2 (<1%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	4 (2%)	20 (12%)	12 (2%)
Normal macro, Histo too autolysed	1 (<1%)	1 (1%)	151 (26%)
Total:	**225**	**163**	**587**

Table 144 Microscopic findings in the pancreas with normal macroscopic findings for different death categories

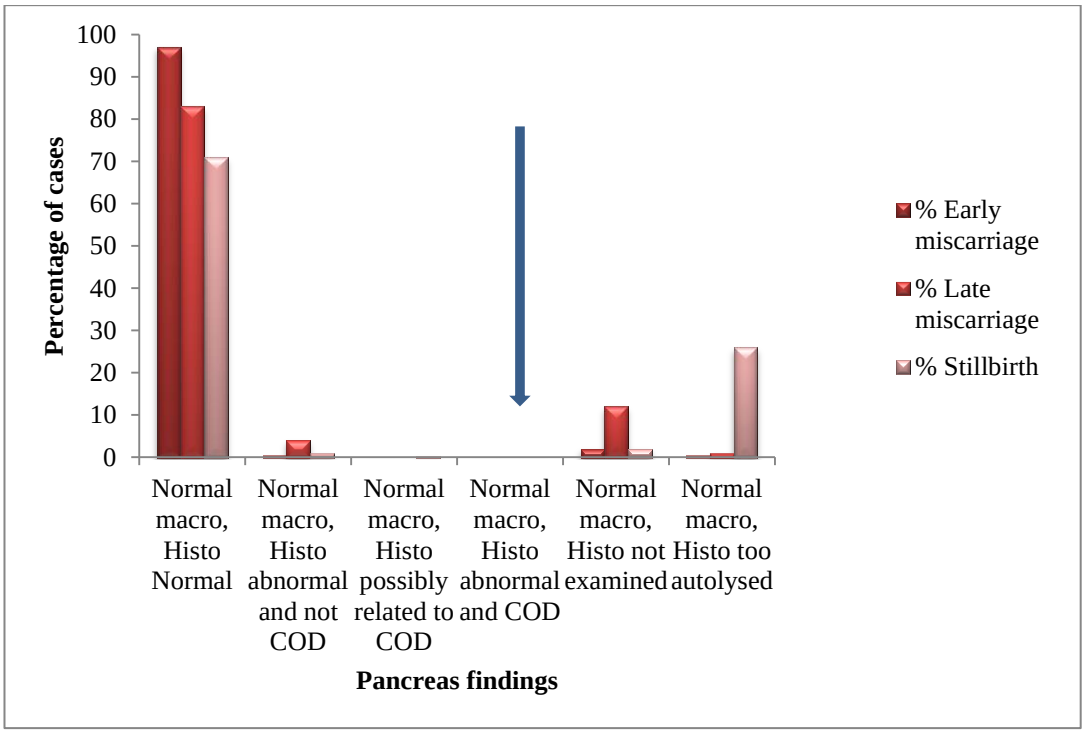


Figure 100 *Microscopic findings in different death categories with normal macroscopic findings in the pancreas*

The majority of cases had both a normal macroscopic and microscopic appearance of the pancreas. There were no cases in which the macroscopic appearance was normal with the microscopic examination providing the definitive cause of death.

Thyroid

Thyroid Macro	Early miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	213 (87%)	144 (80%)	550 (86%)	**907 (85%)**
Abnormal BUT not contributed to death	0 (0%)	0 (0%)	11 (2%)	**11 (1%)**
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	31 (13%)	35 (20%)	78 (12%)	**144 (14%)**
Too autolysed	1 (<1%)	0 (0%)	0 (0%)	**1 (<1%)**
Not given	1	0	0	**1**
Total	**246**	**179**	**639**	**1064**

Table 145 Macroscopic findings in the thyroid for different death categories

The majority of cases had a normal macroscopic appearance of the thyroid gland (85%). Stillbirths had the greatest proportion of macroscopic abnormalities of the thyroid and all were minor changes that did not directly contribute to death. There were no cases in which abnormal macroscopic abnormalities of the thyroid provided a definitive cause of death.

Thyroid Histology	Early miscarriage	Late miscarriage	Stillbirth	**Total**
Normal	182 (74%)	118 (66%)	493 (77%)	**793 (75%)**
Abnormal BUT not contributed to death	0 (0%)	0 (0%)	9 (1%)	**9 (1%)**
Abnormal and potentially contributed to death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Abnormal and definitive cause of death	0 (0%)	0 (0%)	0 (0%)	**0 (0%)**
Not examined	63 (26%)	59 (33%)	132 (21%)	**254 (24%)**
Too autolysed	1 (<1%)	2 (1%)	5 (1%)	**8 (1%)**
Total	**246**	**179**	**639**	**1064**

Table 146 Microscopic findings in the thyroid for different death categories

The majority of thyroid gland histology was normal (75%). Stillbirths had the greatest proportion of microscopic abnormalities of the thymus gland and all were minor abnormalities that did not directly contribute to death. There were no cases in which a microscopic abnormality in the thymus gland provided the definitive cause of death.

Thyroid	Early miscarriage	Late miscarriage	Stillbirth	Total
Normal macro, Histo Normal	181 (85%)	118 (82%)	483 (88%)	782 (86%)
Normal macro, Histo abnormal and not COD	0 (0%)	0 (0%)	7 (1%)	7 (1%)
Normal macro, Histo possibly related to COD	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo abnormal and COD	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Normal macro, Histo not examined	32 (15%)	25 (17%)	55 (10%)	112 (12%)
Normal macro, Histo too autolysed	0 (0%)	1 (1%)	5 (1%)	6 (1%)
Total	213	144	550	907

Table 147 Microscopic findings in the thyroid with normal macroscopic findings for different death categories

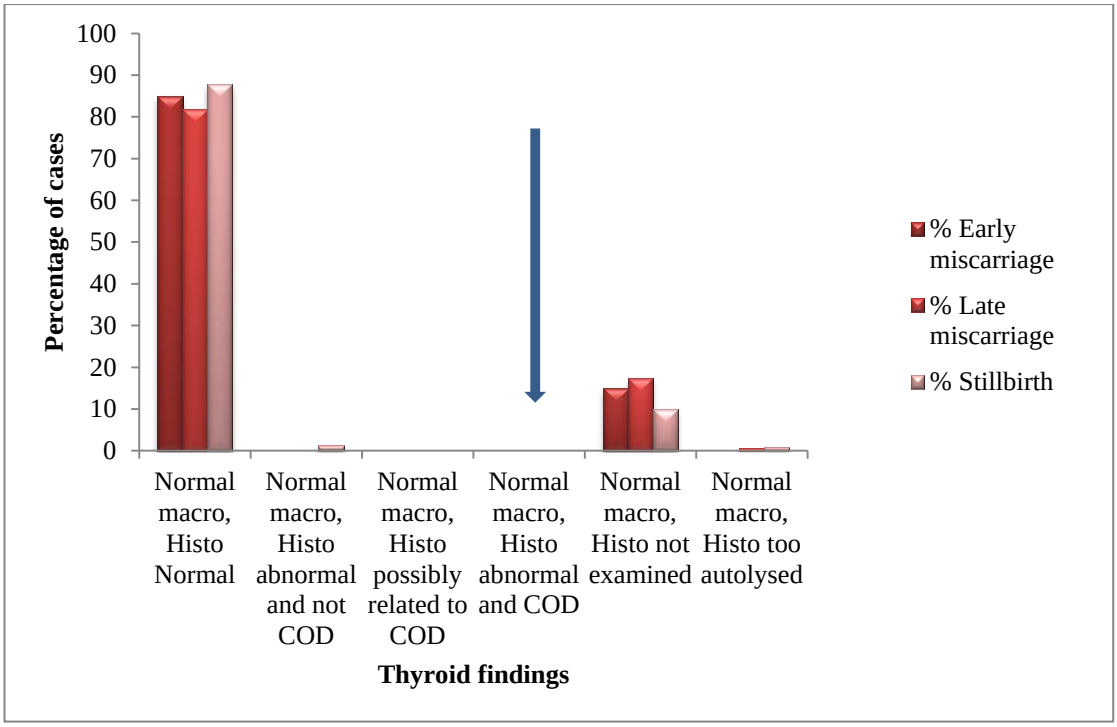


Figure 101 *Microscopic findings in different death categories with normal macroscopic findings in the thyroid*

The majority of cases had both a normal macroscopic and microscopic appearance of the thyroid. There were no cases in which the macroscopic appearance was normal with the microscopic examination providing the definitive cause of death.

8.3 Discussion

The majority of all fetuses in this study had normal macroscopic and microscopic appearances of their internal organs. Cases of stillbirth showed a greater proportion, on average, of abnormal macroscopic or microscopic appearances but almost all were nonspecific and did not provide the cause of death. The same was true for miscarriages (*Table 148 and Figure 102*).

Organ	Abnormal Histology and Cause of death	Abnormal Histology but NOT cause of death	**Total:**
Heart	1 (3%)	58 (4%)	**59 (4%)**
Lung	21 (68%)	489 (34%)	**510 (35%)**
Liver	1 (3%)	118 (8%)	**119 (8%)**
Brain	3 (10%)	301 (21%)	**304 (21%)**
Kidney	5 (16%)	149 (10%)	**154 (10%)**
Spleen	0 (0%)	53 (4%)	**53 (4%)**
Adrenal	0 (0%)	70 (5%)	**70 (5%)**
Thymus	0 (0%)	191 (13%)	**191 (13%)**
Pancreas	0 (0%)	10 (1%)	**10 (1%)**
Thyroid	0 (0%)	7 (<1%)	**7 (<1%)**
Total:	**31**	**1446**	**1477**

Table 148 Histological abnormalities in organs (1 case had histological abnormalities of the liver, the lungs and the brain and another case had abnormalities in the kidney and the lungs; 28 individual cases therefore had abnormal histology that was directly linked to the cause of death) Note: Percentages within this table represent column percentages not row percentages.

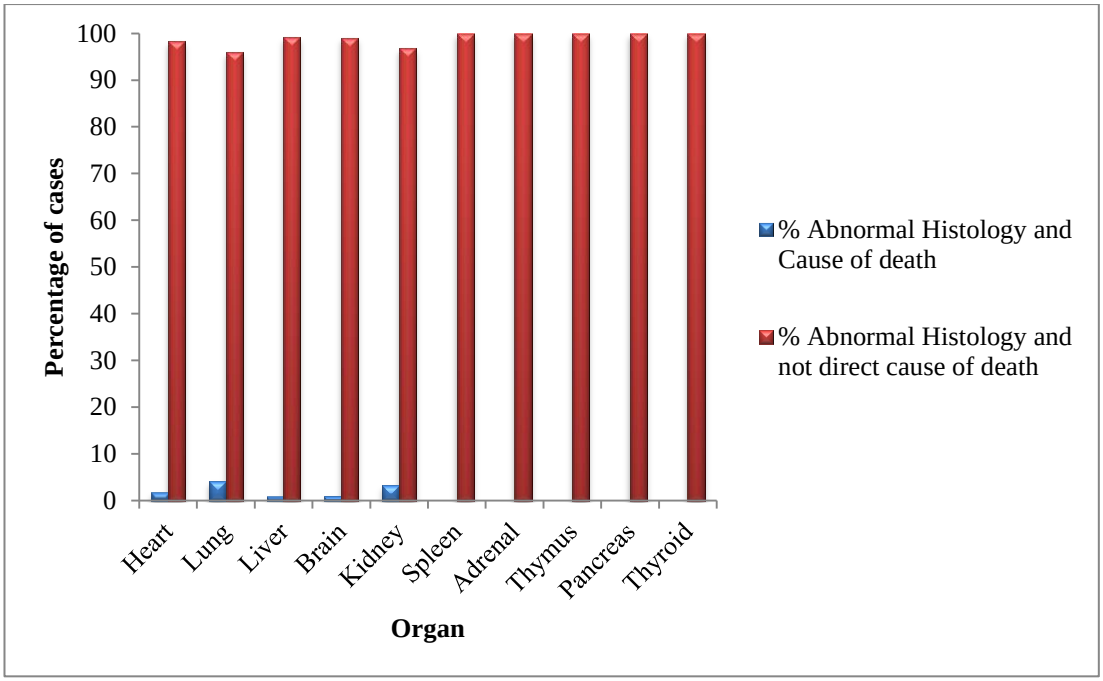


Figure 102 *Comparison in percentage of organ histological abnormalities between those that directly provided the cause of death and those that did not directly provide the cause of death.*

Thirty-one organs had abnormal histological findings that directly contributed to the cause of death. However, one case had histological abnormalities of the liver, the lungs and the brain and another case had abnormalities in the kidney and the lungs therefore 28 individual cases, of more than 1,000 intrauterine deaths, had abnormal microscopic findings that directly provided the cause of death (for the following discussion the two cases with multiple abnormal organs have been placed in the abnormal lung histology group).

The majority of all histological abnormalities (68%) were seen in lung tissue; a similar finding to the histological abnormalities found in cases of Sudden Unexpected Death in Infancy (SUDI) where 43% of histological abnormalities are found in lung tissue (218).

14 cases with abnormal lung histology also had chorioamnionitis on placental histological examination which could have provided the same cause of death. Four cases of ascending infection with abnormal lung histology would most likely also

have had abnormal placental histology, if a placenta had been submitted for examination. Of the remaining ten cases with abnormal histology that directly provided the cause of death there were:

- Two cases of histological abnormalities in the kidneys (thrombi) could have been given the same cause of death through examination of the placenta (Fetal Thrombotic Vasculopathy)
- One heart, one lung and two kidney cases had macroscopically identifiable congenital abnormalities which could have been diagnosed using postmortem Magnetic Resonance Imaging as could the two intracerebral bleeds.
- The two cases with cytomegalovirus (CMV) infection were diagnosed in fetal lung and kidney tissue, but not seen in placental tissue (however one case had no placental tissue submitted for examination), suggesting it was only possible to diagnose the CMV after invasive autopsy in at least one of these two cases.

27 cases with histological abnormalities therefore had the potential to be diagnosed with the same cause of death without the need for microscopic examination of fetal organs. Indeed published work (albeit from small a small study) has found that postmortem MRI, together with investigations such as placental histology, external fetal examination, cytogenetics and plain film radiography can provide the equivalent information to that of a complete invasive autopsy (214). One study, of intrauterine fetal deaths greater than 20 weeks gestation, reported that the most valuable test to help determine the cause of fetal death was examination of the placenta (219). Furthermore, placental examination, without autopsy, has also been

reported to provide a cause of death in nearly 50% of intrauterine, intrapartum and neonatal deaths (220).

There were no cases in the present study in which histology of the spleen, adrenal glands, thyroid, thymus or pancreas provided the cause a death; a finding similarly demonstrated in the histological examination of organs in cases of SUDI (218).

Of the total 1,064 cases there were only 16 cases with organs which had a normal macroscopic appearance but abnormal histology that directly gave the cause of death. All 16 cases have been discussed above; 14 lung abnormalities with ascending infection (nine of which had chorioamniontis in the placenta, one CMV infection and the remaining four had no placenta submitted for examination), and 2 cases of fetal thrombotic vasculopathy with abnormalities in the kidney, which also had abnormalities within the placenta that could have provided the same cause of death. These findings suggest that if fetal macroscopic organ examination is normal, there is very little benefit in histological examination in intrauterine deaths. This differs from results found in the investigation of SUDI, where 26% of lungs, 2% of hearts and 1% of livers had reported normal macroscopic results but significant findings on histological examination (218).

As there are no other large scale published studies providing an in depth review of histological abnormalities at autopsy, it must be recommended that further studies are completed to corroborate these findings; it is clear, however, that routine histological examination of all organs in stillbirth and miscarriage is of limited value in providing the accurate cause of death.

Several caveats should be noted regarding these data and their interpretation. First, this only applies to predominantly structurally normal intrauterine deaths, not

terminations of pregnancy for anomalies, since in such a population, targeted histological sampling may be essential for diagnosis. Secondly, the interpretation here was limited to findings that influenced directly the final cause of death. Therefore, some abnormalities, such as finding subtle brain changes suggestive of ischaemia, may indeed add to our understanding of the process of intrauterine death, but their presence of absence will not alter the final cause of death in the absence of other significant findings such as placental pathology, etc. Finally, it should be recognised that this interpretation is based on routine histological assessment. It is possible that in future, novel investigations may be introduced which require organ specific fetal tissue, such as various omic approaches, which would provide additional rationale for invasive evaluation and sampling.

9. Thymus Histology

9.0 Background

9.1 Methods

9.2 Results

9.3 Discussion

9.0 Background

The thymus is an organ that develops embryologically from the third and fourth pair of pharyngeal pouches and at birth usually weighs between 10-35g (221). The thymus gland can involute in response to either stress or age and involution is more complex than a single negative exponential function of age alone (222). Histologically, involution of the thymus is characterised by karyorrhexis of lymphocytes with active phagocytosis by macrophages – creating the typical "starry sky" appearance of the cortex which then progressively thins until the corticomedullary ratio is reversed (223).

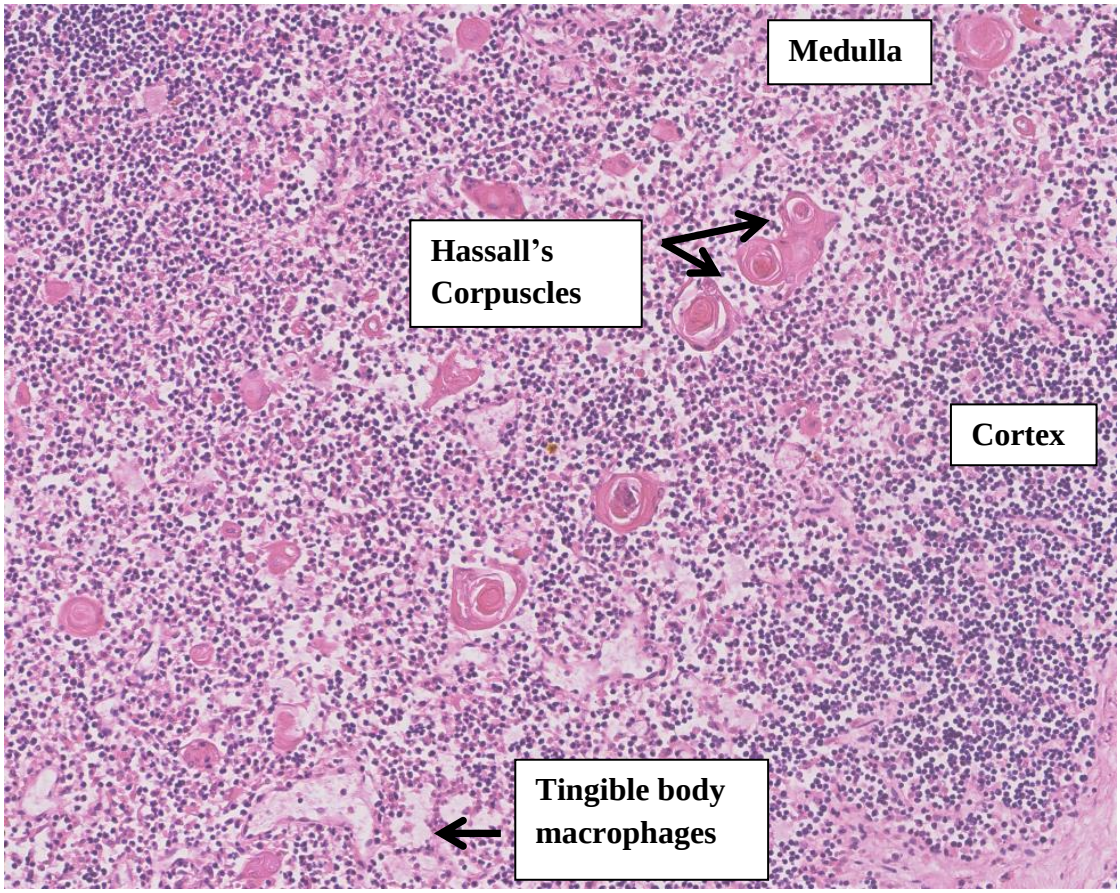


Figure 103 *Thymus gland histology at x10 magnification with evidence of involution*

Histological examination of the thymus gland is typically undertaken in stillbirth autopsies to assess for accelerated involution which has been suggested to be an indicator of prenatal stress (171, 172). Existing data indicates that the degree of

thymic involution varies with many factors such as maternal diabetes mellitus or fetal cause of death such as chorioamnionitis (210, 224, 225). Involution is graded using the Van Baarlen Grading scale (*Figure 104).*

Grade 0: The resting state, ie, a clear cortex-medulla distinction with a high density of lymphocytes in the cortex. The thymic lobules closely packed together and separated only by delicate connective tissue septa. Lymphophagocytosis in the cortex was absent.

Grade 1: Identical to grade 0, except for the presence of some lymphophagocytosis in the cortex.

Grade 2: More pronounced lymphophagocytosis in the cortex, with a "starrysky" aspect. In addition, early signs of shrinkage of the cortex and some separation of the thymic lobules. In other aspects, the histologic picture is similar to that of grade 0 or 1.

Grade 3: Loss of distinction between cortex and medulla at low magnification due to advanced lymphophagocytosis, resulting in irregular narrowing of the cortex, loci of lymphodepletion, and increasing separation of thymic lobules.

Grade 4: More pronounced lymphodepletion of the cortex. This often presents a "reverse" picture, lymphocyte density being higher in the medulla than in the cortex. Most often, the distinction between cortex and medulla is lost. There is a relative prominence of interstitium and blood vessels (perivascular areas). Advanced shrinkage and separation of the thymic lobules observed.

Figure 104 *Van Baarlen Grading of thymic involution (173)*

However, the assessment of thymic involution is essentially subjective and relies on consistent reporting from perinatal pathologists making judgements on the degree of lymphodepletion and shrinkage of the cortex without the use of measurements. Comments regarding thymic histological appearances derived from pathology reports are also unreliable due to the absence of blinding of the pathologist to the clinical features, introducing the potential for significant bias.

Since thymic involution is most often used as a potential marker for fetal IUGR, this chapter aims to assess whether evidence of thymic involution can be performed in a more objective manner and blinded to clinical features and as a result whether evaluation of thymic involution in cases of stillbirth is reliably associated with different causes of death, in particular IUGR. Table 149 highlights current published literature about thymic involution in which the majority only use subjective criteria to assess thymic involution and as such their results are likely confounded by observer bias.

Author	Cases	Methods	Results
Van Baarlen et al.	234 cases (fetuses and young children)	**Age of cases:** 20 weeks gestation to 17 years. **Duration of acute illness**: 0 hours - 12 months. **Grading of thymic involution**: Van Baarlen grading (blinded). **Morphological assessment of thymus:** Projection microscope.	Thymus weight and volume percentages of interstitium, cortex and medulla were significantly related to prenatal or postnatal status and the age of patient. The thymus weight related to duration of illness in prenatal patients. The 5 Van Baarlen gardes were significantly correlated with the duration of acute illness.
Bancalari et al.	30 cases (all neonatal deaths)	**Morphological assessment of Thymus:** Macroscopic and microscopic assessment was correlated with clinical and radiological data.	Malformations, severe asphyxia and hyaline disease showed a moderate decrease in thymus weight and slight depletion of cortical lymphocytes. Newborns with severe infection had

			pronounced decreased thymic weight and severe depletion of cortical lymphocytes. There was no correlation between radiological and postmortem findings.
Agapitos et al.	300 cases (all perinatal deaths)	**Grading of thymic involution:** 5 groups (1. normal -mature thymus, 2. involutionary changes - "starry sky", 3. involutionary changes - intense lymphocyte depletion, 4. hypoplasia - aplasia, 5. agenesis). **Classification of deaths:** Wigglesworth classification system	A strong correlation was reported between thymic weight and gestational age, birth weight and histological features as well as histological features of the thymus and cause of death.
Toti et al.	50 cases (30 cases with chorioamnionitis / sepsis; 20 controls)	**Age of cases:** 16 weeks gestation to term. **Grading of Thymic involution**: 1-3 (1: resting state; 2: early signs of shrinkage, some separation of thymic lobules and relative prominence in interstitium and blood vessels, cortex partly devoid of lymphocytes; 3: extensive lymphodepletion, irregular narrowing of the cortex (sometimes completely disappeared) (blinded) **Morphological assessment of thymus:** Non- parametric test point analysis at low and high power fractal analysis and immunohistochemistry	Cases of chorioamnionitis showed decreased thymic volume, reduced corticomedullary ratio, significant changes in the relationship between thymic parenchyma and thymic insterstital tissue with resulting organ complexity, severe reduction in thymocytes and other degenerative processes such as monocyte/macrophage infiltration of Hassall's Bodies. The corticomedullarty ratio in controls was reported as 1.46-3.46.

			The main conclusion drawn was that chorioamnionitis with or without sepsis was associated with significant morphological changes of the thymus.
Glavina-Durdov et al.	100 cases (perterm and term fetal deaths)	**Age of cases:** 21-41 weeks gestation with death ranging from <1 hour to 28 days after birth. **Grading of Thymic involution:** Van Baarlen grading system.	25 of 38 neonates who lived <12 hours had a Grade 0 Thymus. In 13 of the 38 neontaes who lived < 12 hours involution suggested prenatal stress. The grade of thymic involution significantly related to duration of illness. Placental inflammation was significantly associated with thymic involution and pre-term newborns with infection were significantly more associated with acute thymic involution than cases of respiratory distress. A significant increase in peripheral blood lymphocyte count in grade 0-2 was reported and a decrease in peripheral blood lymphocytes in grades 2-4.
Edwards et al.	23 cases (matched pairs of stillbirths)	**Age of cases:** 24 - 41 weeks gestation. **Type of maternal**	In maternal diabetes the thymus was small with a starry sky appearance in 11 of

		Diabetes: Any form of diabetes mellitus or no diabetes mellitus. **Morphological assessment of thymus:** Cortical lymphocyte depletion and hassalls corpuscles used as markers of hypoxic stress.	20 cases compared to 4 of 22 in controls and these findings were associated with a low placental weight.
Jacques et al.	10 cases (8 stillbirths; 2 neonatal deaths)	**Age of cases:** Third trimester fetuses that died during or very close to their time of delivery. **Grading of thymic involution:** Van Baarlen grading system.	Recent brain injury was found in nine cases. Thymic involution was present in eight cases; five mild and three severe (grade 3/4). Myocardial infarcts were reported in two cases, intrathoracic petechiae in five cases and ascites or pleural effusions in six cases. Severe thymic involution and myocradial infarcts correlated with established brain injury. Increased nucleated red blood cells or villous hypervascularity was reported in five cases and correlated with established brain injury. Acute chorioamnionitis with funisitis was present in one case and chronic inflammatory

			placental lesion in six cases.

Table 149 Summary of published literature about Thymic involution. (171-173, 210, 224-226)

9.1 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriage from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Three different groups of cases were selected for further thymic analysis and gathered using Microsoft Access queries and Microsoft Excel. The three groups included;

1. *Controls (20 cases)*: Unexplained unexplained deaths with no associated findings at autopsy, of normal birthweight and with no abnormalities of the placenta (either definitive or of uncertain significance).
2. *IUGR (14 cases):* Cases that were either known antenatal IUGR or cases found to be SGA based on delta birthweight values (Chapter 5) all of whom also had significantly abnormal placentas with evidence of maternal vascular malperfusion.
3. *SGA (12 cases):* Cases with no history of IUGR but found to be SGA based on delta birthweight values (Chapter 5) all of whom showed no placental abnormalities (likely physiological SGA rather than pathological IUGR)

46 cases in total were selected for analysis. There was no statistical difference in the gestational age of the fetuses between each group. Each case had a 4 micrometer Haemotoxylin and Eosin stained microscopic slide of the thymus gland that was then digitally scanned using the NanoZoomer Hamamatsu Scanner (Digital Pathology

Software; NDP. Scan 2.5, NDP.view2). The slide scanner allowed for good quality images to be obtained of each entire case for subsequent independent analysis of the microscopic features. Each case was recorded using a study number assigned to the case, which allowed cases to be blinded during analysis but identified at the end of the study.

Sections were objectively assessed over four different representative areas for changes described in thymic involution. These included:

1. *Corticomedullary ratio:* A representative lobule was measured in square millimeters in one field of view at x2 magnification in each case. The medulla was then measured in the same way. The area of the medulla was subtracted from the area of the lobule to calculate the area of the cortex. The corticomedullary ratio (of the area) could then be calculated.
2. *Number of Hassall's Corpuscles:* The number of corpuscles were counted in six randomly selected fields at x4 magnification in each case.
3. *Distance between lobules:* The distance was measured in millimeters between two lobules in six randomly selected fields at x4 magnification in each case and an average distance between lobules was calculated.
4. *Number of tingible body macrophages:* The number of tingible body macrophages were counted in one representative high power field in each case.

The cases were also graded subjectively using the Vann Baarlen grading system by a single pathologist. During the examination of images the cases were blinded and only unblinded for statistical analysis of differences between groups. Statistical tests

were completed in Graph Pad Prism and Stats Direct and can be viewed in detail in Appendix 3.

9.2 Results

A complete table of all measurements taken can be found in Appendix 6. When comparing the control group to the IUGR/SGA group, or the IUGR versus SGA groups there were no significant differences in:

1. The number of Hassall's corpuscles per field (p= 0.9931, p=0.483, p=0.486).
2. The number of tingible body macrophages per field (p=0.9638, p=0.3021, p=0.3266).
3. The average distance between lobules (p= 0.6165, p=0.6939, p=0.9798)

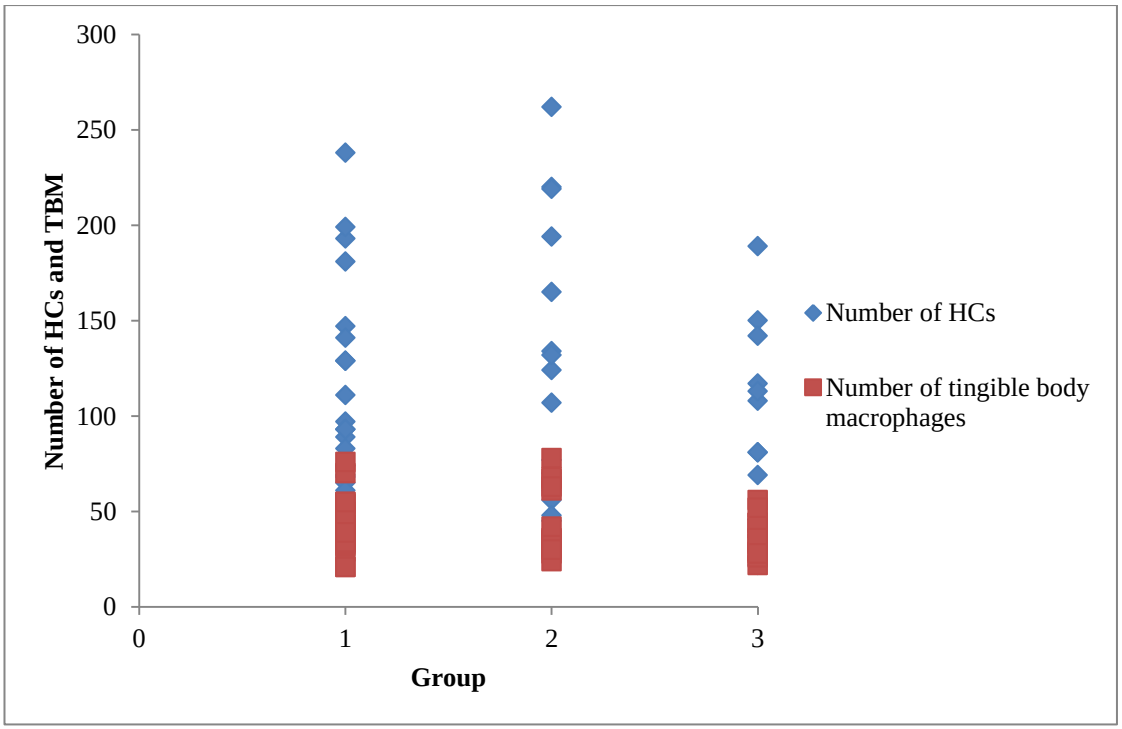


Figure 105 *Number of Hassall's Corpuscles (HCs) and Tingible body macrophages (TBM) in each group where group 1 is the control group, group 2 is IUGR with abnormal placentas and group 3 is SGA with normal placentas. No significant differences were found between the groups.*

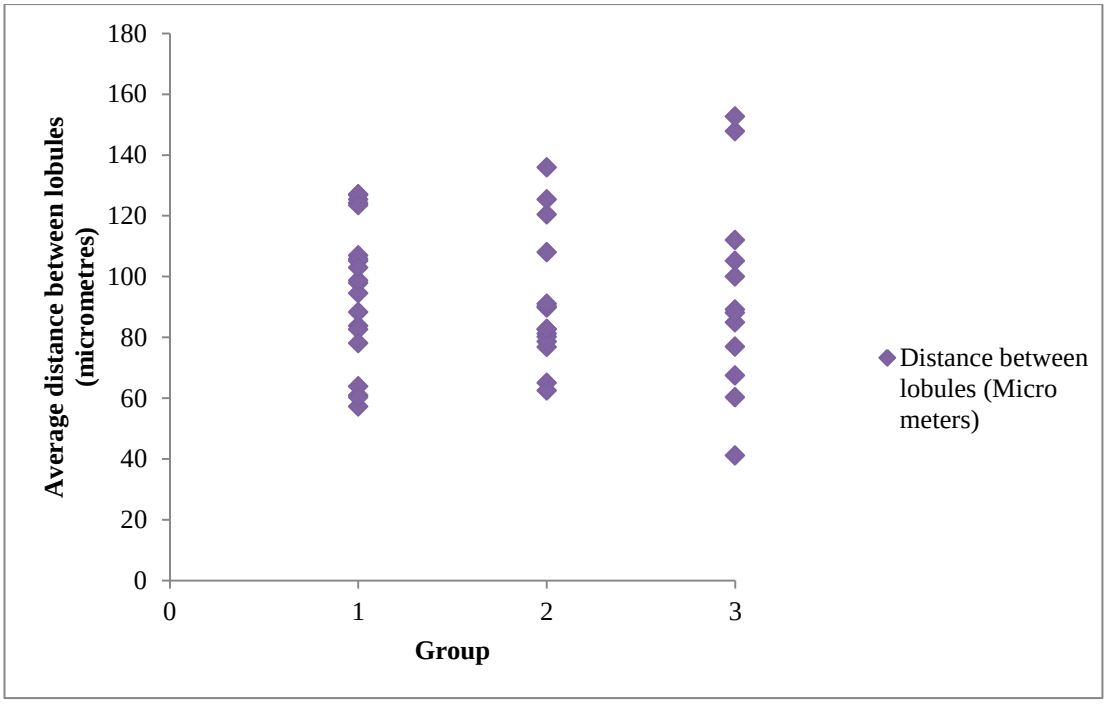


Figure 106 *Distance between lobules (micrometres) in each group where group 1 is the control group, group 2 is IUGR with abnormal placentas and group 3 is SGA with normal placentas. No significant differences were found between the groups.*

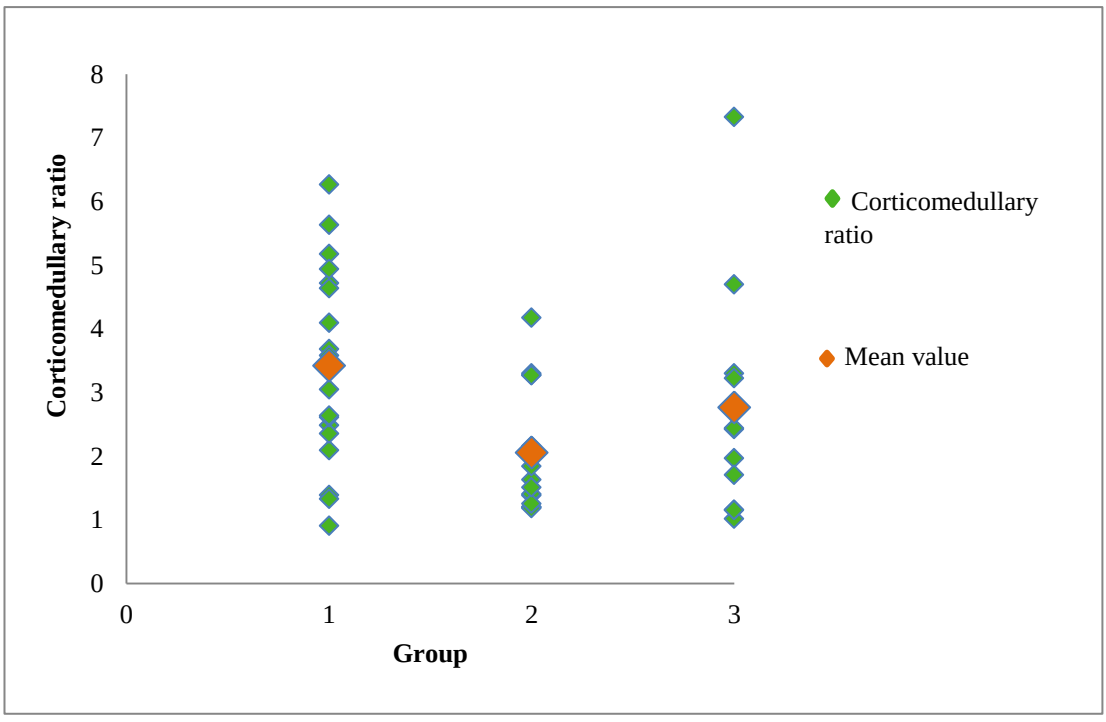


Figure 107 *Corticomedullary ratios (square mm) in each group where group 1 is the control group, group 2 is IUGR with abnormal placentas and group 3 is the SGA with normal placentas. IUGR cases with abnormal placentas (group 2) had significantly smaller corticomedullary ratios than controls (group 1) (p= 0.0221). (Excludes 4 cases, 1 of which was too autolysed for assessment and 3 were too high grade to differentiate the cortex from the medulla)*

The only significant finding was that cases with IUGR had significantly reduced corticomedullary ratios than controls indicating a significantly smaller cortex (p= 0.0221: Median 1.63 (range 1.18-4.17) versus median 3.32 (range 0.91 – 6.27), median difference -1.24 (95% CI -2.38 to 0.20)). However, using ROC statistics the most useful cut off of corticomedullary ratio in the differentiation of cases with IUGR and controls is 2.13 with a sensitivity of 64% and specificity of 78% (*Table 150*). However, it must be noted that the distributions of corticomedullary ratios in all three groups overlap massively and therefore using a specific ratio, as a diagnostic tool in IUGR, is likely to be inaccurate.

Corticomedullary ratio Cut Off	Sensitivity %	Specificity %
1.05	0	94
2.13	64	78
3.16	71	50
4.13	92	33
5.06	100	17

Table 150 Sensitivity and specificity for the differentiation of IUGR against control cases using specific corticomedullary ratio cut offs.

There was no difference in the corticomedullary ratio between normal controls and the SGA group, supporting the concept that stillbirths with body weight below the 10th centile but no placental features of maternal vascular malperfusion are likely artefactually or physiologically small and do not indicate pathological IUGR as the likely cause of death.

9.2.1 Van Baarlen Grading

Van Baarlen Grade	Controls	IUGR Abnormal placenta	SGA Normal placentas	**Total**
1 (Low)	1 (5%)	0 (0%)	1 (8%)	**2 (4%)**
2 (Low)	12 (60%)	9 (64%)	9 (75%)	**30 (65%)**
3 (High)	6 (30%)	3 (21%)	2 (17%)	**11 (24%)**
4 (High)	1(5%)	2 (14%)	0 (0%)	**3 (7%)**
Total:	**20**	**14**	**12**	**46**

Table 151 Number and percentage of cases that were assigned each Van Baarlen Grade.

The majority of cases (65%) had a low Van Baarlen grade and there were no significant differences in the distribution of low and high grade cases between any of the groups. Furthermore, within each of the separate groups, there were no significant differences in the number of cases that would be expected (by chance) to have a low or high Van Baarlen Grade and those that were observed to have a low or high Von Baarlen Grade (p=0.3373, 0.3625 and 0.083). These finding suggest that the Van Baarlen grading method is no more accurate at differentiating the degree of thymic involution than if chance alone was to assign the degree of involution and that the grading system does not help differentiate between different mechanisms or causes of death in stillbirth when assessed blindly. Importantly, there is a marked variation in grade even in controls, making the test of limited value in clinical practise.

9.3 Discussion

This chapter aimed to assess whether thymic involution could be objectively assessed and used as a tool to help provide a definitive cause of death in stillbirth, in particular to distinguish IUGR due to maternal vascular malperfusion form other causes. The results demonstrate that, when assessed blinded to all clinical history

from scanned images, there was no significant difference in the number of tinigible body macrophages, Hassall's corpuscles or the average distance between lobules between groups. There was a significant statistical reduction in coricomedullary ratio in the IUGR case group, but with extensive overlap of all distributions. Furthermore, the Van Baarlen Grading system, when assessed blindly, was not associated with and particular cause of death group in stillbirth.

These findings are in keeping with the suggestion that overall, with typical IUGR there may be slightly reduced overall thymic size and weight (see Chapter 6) with increased thymic involution, as evidenced by reduced corticomedullary ratio but subjective assessment is of limited value as a reliable tool to help determine the level of intrauterine "stress" and therefore cause of fetal demise, in individual cases due to the large overlap in distributions.

There were some technical difficulties in assessing these thymic microscopic features which should be mentioned. Firstly, some of the images, despite the use of a high quality digital scanner, were of poor quality (compared to traditional microscopy slide assessment) for high power assessment of tingible body macrophages and Hassall's corpuscles. Secondly, in some high grade cases the distinction between the cortex and medulla was indistinct, making corticomedullay ratio calculation difficult. Lastly, when trying to assess the distance between lobules the use of a 2D image of a 3D structure means that the true angle between lobules may vary, with the effects of distance; again this applied equally to all cases.

Chapter 6 in this thesis examined the relationship between the Body:Thymus weight ratio, fetal SGA and cause of death. Results showed significant differences between SGA and non SGA groups and SGA cases with and without placental causes of

death. A Body:Thymus weight ratio of 855.5 could be used to differentiate "true" IUGR from SGA cases without significant placental pathology with 50% sensitivity and 75% specificity. Furthermore, a Body:Thymus weight ratio cut off of 671.7 could be used to define "true IUGR" from all other causes of death with a sensitivity of 61% and specificity of 79%.

In conclusion, these findings demonstrate that there is evidence for statistical reduced thymic size and "involution" in stillbirth with IUGR and maternal vascular malperfusion using blinded morphometric assessments. However, due to the large overlap of distributions, variation within groups, and difficulties of subjective evaluations, the Van Baarlen grading system is an inaccurate tool in the assessment of thymic involution and this assessment is of limited use in clinical practise.

10. Proteomics of formalin fixed, paraffin embedded archival material from stillbirths

10.0 Background

10.1 Methods

10.2 Results

10.2.1 The placenta

10.2.2 The Liver

10.3 Discussion

10.0 Background

Proteomics is the study of the protein complement of samples such as body fluids or tissues; their identification; modification; quantification and localisation (227). Developments in protein ionization methods have facilitated the use of mass spectrometry in proteomic analysis, and this is now the main method in use (228). A mass spectrometer is composed of an ion source, an analyser that measures mass-to charge ratio (m/z) and a detector that recognizes the number of ions at each m/z value (228). Liquid-based chromatography, used in conjunction with electrospray ionization (ESI) can be used to produce ions from a solution of protein extracted from tissues, which is then analysed by mass spectrometry for mass and quantity determination (227-229).

Two different approaches can be used to prepare proteins for mass spectrometry analysis. Firstly, bottom-up (or shot-gun) proteomics is a technique in which proteins are digested (usually with Trypsin) prior to mass spectrometry analysis and the resulting peptide masses are used to sequence and identify all specific proteins present in the sample (227, 230-232). Alternatively, top-down proteomics uses intact proteins and their fragments to identify specific proteins in the sample (227).

It was long assumed that formalin fixed paraffin embedded (FFPE) human tissue was too problematic for use in proteomics due to issues with formalin-induced cross-linking and modifications (*Figure 108*). However, with technical advances it is now possible to reliably derive protein form such tissue (233-238).

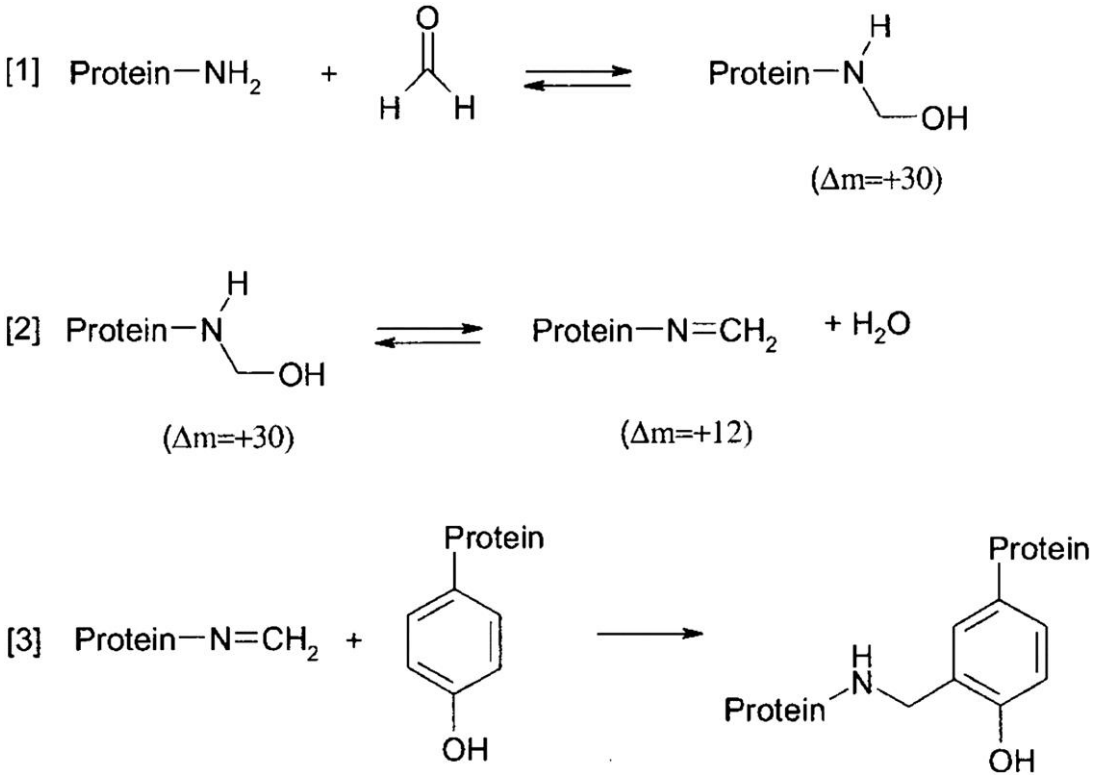


Figure 108 *Reaction mechanism for formaldehyde cross-linking of proteins. Reaction [1] creates a hydroxymethyl-methylol adduct with a + 30 Da mass shift. Reaction [2] is a water elimination to form a Schiff base and a total + 12 mass shift. Reaction [3] is a cross-linking reaction where nucleophilic attack on the methylene carbon of the Schiff base by a nucleophile in the amino acid residue results in a methylene bridge (238). (This research was originally published in Journal of Biological Chemistry (239))*

However, there are no published studies which examine the potential use of fetal FFPE tissue from intrauterine deaths for use in proteomics. The primary aim of this Chapter is to therefore explore the feasibility of extracting protein from routine archival FFPE samples of fetal tissue, from cases of stillbirth, and therefore assess the possible use of proteomics as a future investigative tool in stillbirth causation and clinical investigation. If applicable, the use of a validated database with well-characterised populations of intrauterine deaths, such as from the present study, will allow identification of numerous distinct subgroups for further targeted analysis and hence provide a resource for future studies of stillbirth mechanisms.

10.1 Methods

The Microsoft Access Autopsy Database was used to collate postmortem and antenatal details available for all stillbirths, early and late miscarriage from 2005 – 2013 from Great Ormond Street Hospital and St George's Hospital, London. Queries were run through Microsoft Access and Excel to establish a list of cases (consented for research) that had chroioamnionitis with and without funisitis on histological examination of the placenta but normal liver histology and a separate group of control cases of unexplained deaths with normal histology of the placenta and liver. The Haematoxylin and Eosin stained microscopic slides were assessed for any tissue autolysis and their paraffin blocks were reviewed to ensure there was adequate material available for proteomic sampling. Using complex laboratory techniques and mass spectrometry (detailed below), proteins were extracted and analysed from formalin fixed placental and liver tissue from each case. Such cases were chosen to represent clear and definite pathologies regarding cause of death in whom no histological liver changes are reported, to determine proof of principle that liver proteomic assessment could be used to determine mechanism of death.

10.1.1 Laboratory Methods

Case Selection

Following formalin fixation and paraffin embedding, 50 microns of tissue was obtained (five rolls of tissue, 10 microns thick were cut) and stored in eppendorfs. The samples were cleaned with incubation in heptane, followed by protein solubilisation with 300mM of Tris-HCL, 2% Sodium Dodecyl Sulfate and 2-Mercaptoethanol, heated to 100 ^{0}C for 20 minutes and then 80 ^{0}C for 2 hours. Following centrifugation at 4^{0}C for 20 minutes, the supernatant was removed with subsequent protein quantification with a modified BCA assay (Pierce™ BCA Protein Assay Kit, ThermoFisher Scientific, UK).

Protein precipitation and preparation for 1D-PAGE

From each sample, 100ug of protein was obtained from each sample and precipitated out into a pellet with chloroform/methanol (at a ratio of 1:3). The samples were washed with ethanol and then the centrifuged at 9000g for 2 minutes. The pellet was air-dried briefly and then re-suspended in 20ul of SDS-PAGE buffer, 10ul of DTE (300mM in H2) and 20ul of H20. The sample was alkylated with 3ul of iodoacetamide (36mg in 1 ml of H_2O) for 45 minutes followed by addition of an extra 20ul of SDS-PAGE buffer to make a final volume of 60ul. 30ul (approximately 50ug) of sample was loaded into a well of an Any kD™ Mini-PROTEAN TGX™ precast protein gel (Bio-Rad Laboratories, UK), along with a full range rainbow marker (Amersham ECL PLex™fluorescent Rainbow Marker, GE Healthcare Life Sciences, UK). Isoelectric focusing was carried out using the Mini-PROTEAN Tetra Cell system (Bio-Rad Laboratories, UK) at 200V for 30 minutes.

Protein staining and in-gel digestion

The gels were stained with 0.1% coomassie, 40% methanol and 7.5% acetic acid before being counter stained with 40% methanol and 7.5% acetic acid. From each lane, the gels were divided into two gel bands and placed into eppendorfs. The coomassie stain was removed with 50% methanol and 0.1% acetic acid. Gel bands were washed twice with 50mM ammonium bicarbonate at ph7.8 before being dehydrated in acetonitrile and then in a speed vacuum (Eppendorf Concentrator Plus™, Eppendorf, UK) for 30 minutes. Protein digestion was undertaken with 60ul of 25ng/ul of sequencing grade modified porcine Trypsin (Promega, UK), reconstituted in 50mM ammonium bicarbonate, pH 7.8 with subsequent incubation at 37 ^{0}C for 12 hours.

Digested peptides were extracted from the gel bands using washes of 1% formic acid, followed by extraction with 50% acetonitrile and 1% formic acid. To this final wash, 1% formic acid was added and the final supernatant was freeze-dried and then reconstituted in 50ul of 3% acetonitrile with 0.1% formic acid.

Electrospray QTOF MS analysis

The samples were analysed with a SYNAPT G2-Si HDMS Q-TOF mass spectrometer (mass acquisition range of 50 to 2000 Da), coupled to a nanoACQUITY UPLC system via a nanospray (ZSpray™) source (SYNAPT, nanoACQUITY and ZSpray from Waters Ltd, UK).

19ul of each sample was vialed and spiked with 1ul (1nmol/ml) of *S. cerevisiae* yeast enolase (Sigma-Aldrich, UK)). 1ul of sample was loaded onto the nanoAcquity column (ACQUITY UPLC™ Peptide BEH C18 nanoACQUITY Column 10K psi, 130A, 1.7µm, 75µm x 100mm, Waters Ltd, UK). The samples were analysed over 60 minutes, utilising a linear gradient of mobile phase A (H_2O with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid) with the initial gradient changing from 3 to 40% of mobile phase B over 40 minutes, followed by 40 to 85% for 2 minutes and then a subsequent decrease to 3% over 1 minute and for the remainder of the run, with a flow rate of 300nL/minute.

The peptides were analysed in positive mode electrospray ionisation with data collected through alternating 1.5 second scans of low (4eV) or high (15-40eV) collision energies. Post-calibration of data files were corrected using the doubly charged precursor ion of [Glu1]--fibrinopeptide B (785.8426 m/z).

Data analysis

All data was processed and analysed using Progenesis QI for Proteomics software 2.0 (Nonlinear Dynamics, UK). Fragment ions in the first 5 minutes and the last 5

minutes of each run were excluded. Protein identifications were obtained by searching the *Homo sapiens* proteome database (including canonical sequences and isoforms) from Uniprot (obtained on 01/03/2015 from www.uniprot.org), to which the sequence of yeast enolase and porcine trypsin was added manually. The spectra obtained from each sample was analysed using the following search parameters; the enzyme was trypsin (up to 2 missed cleavages), fixed modifications of carbamidomethylation of cysteine and variable modification of oxidation of methionine. Peptide and protein matching required two fragment ions per peptide, three fragment ions per protein and more than one peptide per protein. Quantification was performed using the spiked standard of yeast enolase (*S. cerevisiae*). The false detection rate was 1%

Protein function and pathway analysis was performed using the PANTHER (Protein Analysis Through Evolutionary Relationships) classification system.

10.2 Results

As a proof of principle study only, seven cases were selected for proteomic analysis with research consent. Cases analysed included liver and placental samples from:

- Two cases of Chroioamnionitis with funisitis (fetal systemic response) and normal liver histology
    - Gestation: 22 and 23 weeks
    - Intrauterine Interval: 1 day and 0 days respectively
    - Postmortem Interval: 8 days and 10 days respectively
- Two cases of Chorioamnionitis without funisitis (no fetal systemic response) and normal liver histology
    - Gestation: 20 and 22 weeks

  - Intrauterine Interval: 1 day for each case
  - PostMortem Interval: 7 days for each case
- Three control cases of unexplained deaths with both normal placental and liver histology
  - Gestation: 17, 20 and 22 weeks
  - Intrauterine Interval: Uncertain, 0 days and 1 day respectively
  - Postmortem Interval: 13 days, 2 days and 3 days respectively.

10.2.1 The Placenta

Using the methods above, in total, 305 unique proteins were extracted from placental tissue, demonstrating that protein can be successfully extracted from FFPE placental tissue in cases of stillbirth with varying mechanisms of death (Appendix 7).

A wide variety of proteins were obtained including 25 different classes of protein (*Figure 109*) and proteins with nine different types of molecular functions (*Figure 110*).

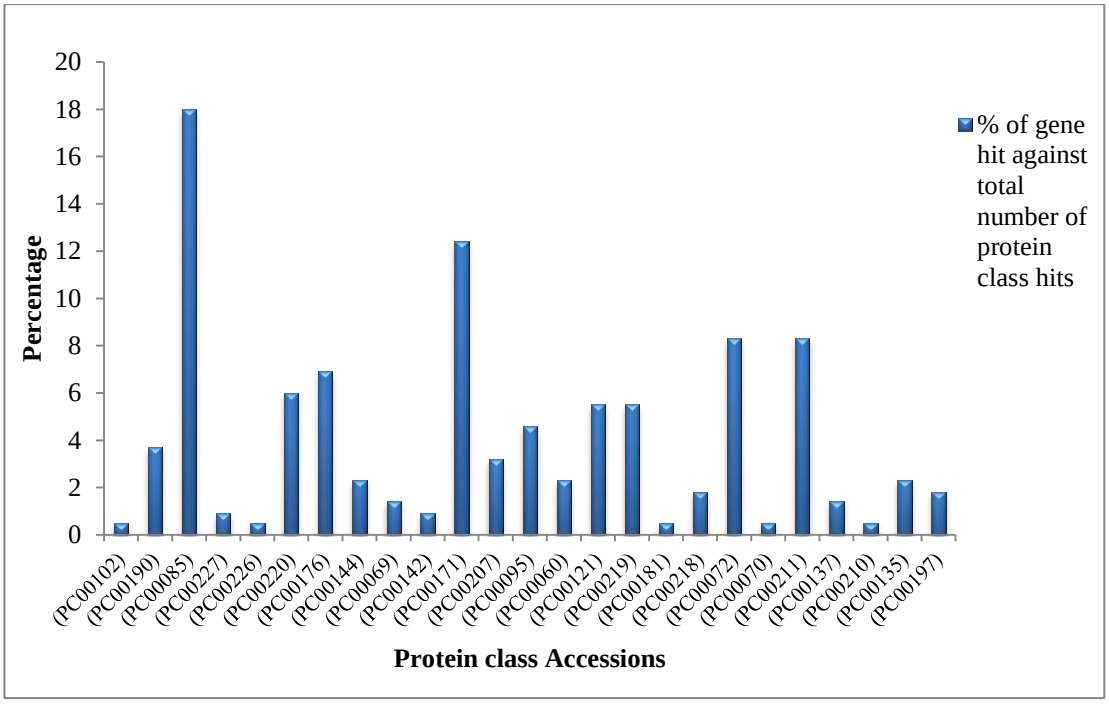


Figure 109 *25 different classes of proteins found of which cytoskeletal protein (accession PC00085) had the greatest percentage of gene hits against protein class hits (18%)*

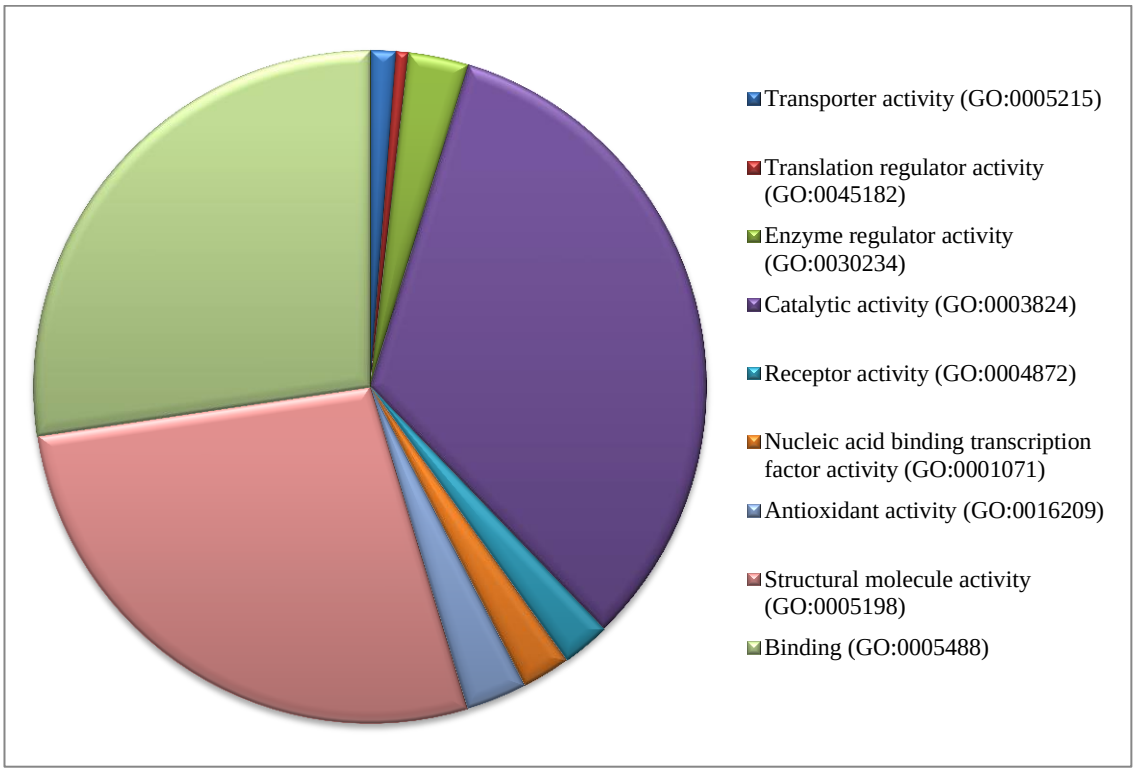


Figure 110 *Proteins with nine different molecular functions were found of which Catalytic activity had the greatest percentage of gene hits against the total number of function hits (32.9%)*

Four proteins were present in significantly different amounts between the case (funisitis and no funisitis) and control groups, despite peptide counts being small (*Table 152).*

Protein	Peptide count	Unique peptides	Anova (p)	Highest mean condition	Lowest mean condition
Peptidyl-prolyl cis-trans isomerase B OS=Homo sapiens GN=PPIB PE=1 SV=2	15	12	0.004	No Funisitis	Funisitis
Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2	15	10	0.01	Funisitis	No Funisitis
Probable phosphoglycerate mutase 4 OS=Homo sapiens GN=PGAM4 PE=2 SV=1	5	3	0.02	No Funisitis	Funisitis
40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2	3	3	0.03	Control	No Funisitis

Table 152 Proteins found to be present in significantly different amplitudes between case groups during proteomic analysis in placental tissue.

These findings suggest that protein expression differs more significantly between cases of Chorioamnionitis with and without funisitis than between control cases and cases of Chrorioamnionitis. This is also highlighted by the differences in normalized abundance between the three different case groups (*Figure 111*).

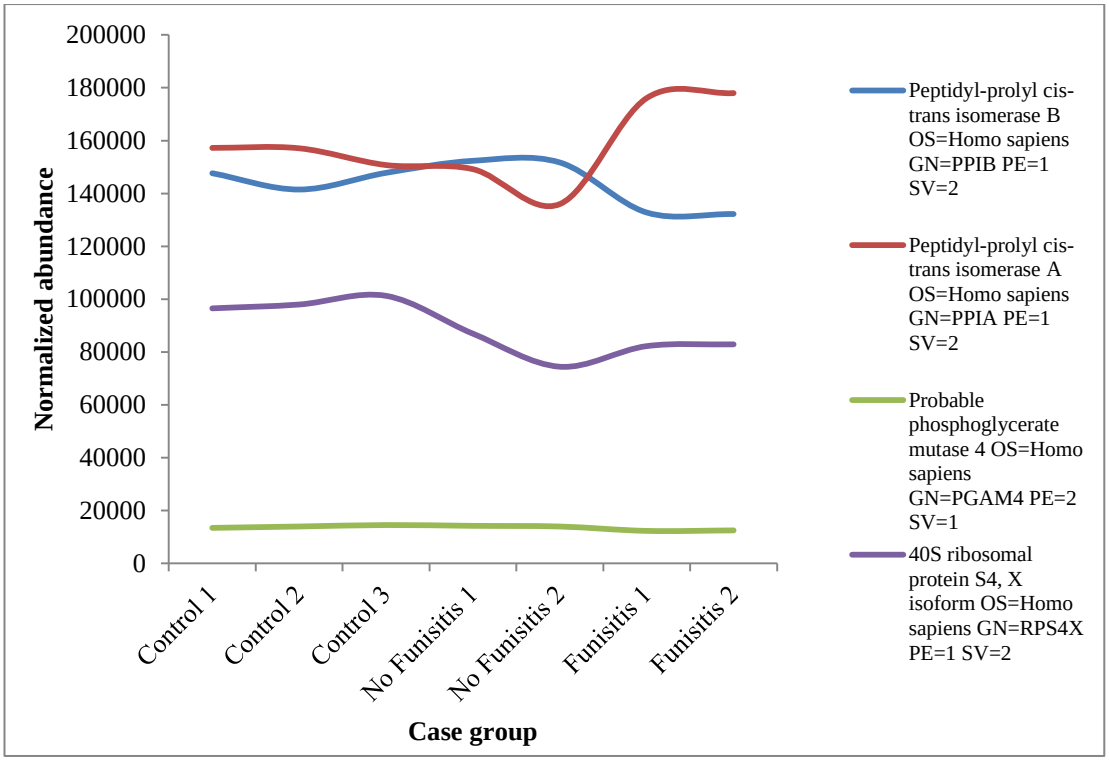


Figure 111 *Normalized abundance of proteins with significantly different amplitudes between case groups*

10.2.2 The Liver

Using the methods above, 250 unique proteins were extracted from the liver tissue. There were 25 different protein classes of which nucleic acid binding (accession PC00171 was the most dominant (15.7%) and 9 different molecular functions, of which catalytic activity was similarly the most prominent (32.6%).

Two proteins were present in significantly different amounts between the case groups, despite peptide counts being small (*Table 153).*

Protein	Peptide count	Unique peptides	Anova (p)	Highest mean condition	Lowest mean condition
3-ketoacyl-CoA thiolase, peroxisomal	10	1	0.043553	Chorioamnionitis	Funisitis
Neurofilament medium polypeptide	9	8	0.041746	Funisitis	Control

Table 153 Proteins found to be present in significantly different amplitudes between case groups during proteomic analysis in liver tissue.

10.3 Discussion

Results from this chapter have demonstrated that it is possible to extract proteins from routinely collected, archival FFPE stillbirth tissue, both fetal and placental, and that there are significant differences in protein amounts between different case groups and in different organs.

In total, 305 proteins were successfully extracted from FFPE placental tissue and 250 proteins from liver tissue however, some of the proteins extracted in this small study could not be identified which could be secondary to the process of maceration, autolysis of tissue or post-translational modifications (i.e. the process of glycosylation of proteins within the endoplasmic reticulum (240)). Four placental proteins and two liver proteins were expressed in significantly different amounts between case groups. However, studies which used fresh tissue with multiple fractions have found yields of up to 4000 proteins and 37 - 171 significantly different proteins between case groups and controls (in cases of intrahepatic cholestasis and pre-eclampsia) (241-243). This highlights the continued complexity of extracting protein from FFPE and as such fresh frozen tissue remains the gold standard for use in clinical proteomics at the present time (244).

The findings of this chapter do however prove, in principle, that proteins can be derived from fetal tissue in stillbirth and that there are some significant differences between cause of death groups. This could be an important step in the investigation of cause of death in stillbirth; if proteomics can highlight cases of infection (for example) that may have been indistinguishable from "unremarkable" cases microscopically, we may find that previously unexplained causes of death have a pathological cause, particularly with regard to "ischaemic" mechanisms. Future work

needs to further develop proteomic techniques with larger scale studies and more diverse case groups using both fresh and FFPE fetal tissue.

11. Overview

11.0 Conclusions

11.1 Limitations and Criticisms

11.2 Evidence based guidance

11.3 Future Studies

11.0 Conclusions

Maternal demographics

Known associations to stillbirth and miscarriage were confirmed such as increasing maternal age, increased maternal BMI (with raised maternal BMI being associated with both miscarriage and stillbirth), maternal ethnicity, diabetes mellitus and hypertension, allowing for comparison of the results within this study to other stillbirth and miscarriage research. This chapter also highlighted that white mothers were significantly overrepresented in stillbirth and black mothers were significantly overrepresented in miscarriage. Mothers who were primigravida were also found to be overrepresented in the stillbirth group however; mothers with an obstetric history of vaginal bleeding during pregnancy, uterine fibroids and in vitro fertilization were overrepresented in miscarriages.

Cause of Death

Two thirds of the study population had an unexplained cause of death using strict criteria applied for this project: 27% of these deaths had no associated clinical, fetal or placental lesions. Black and Asian ethnicities were found to be significantly more associated with ascending infection in miscarriages than white mothers. Mothers over the age of 40 years had significantly more deaths associated with placental pathology than younger mothers. Fetuses with unexplained death were significantly more macerated than non-macerated fetuses suggesting a possible association between the degree of maceration and the ability to assign a specific cause of death. Re-classifying deaths using the ReCoDe classification system found that 36% of cases were SGA and 35% were unexplained, highlighting the difficulty of comparing classification systems. Internal examination alone provided a definitive cause of death in only 1% of cases; 19% of the overall causes of death could have been diagnosed from review of the clinical circumstances and a further 18% by placental

macroscopic and microscopic examination. However, 62% of deaths remained unexplained, around half of whom were associated with either maternal risk factors or placental/histological changes of uncertain significance, suggesting current autopsy practise is of limited utility to identify causes of death and newer approaches are required for more reliable death investigation. There is great variation in cause of death allocation according to subjective interpretation of findings of uncertain significance.

Intrauterine Growth restriction and Small for Gestational age fetuses

Around 35% of stillbirths were SGA based purely on unadjusted birthweight centiles. The majority of SGA fetuses had no identifiable cause of death at autopsy and of the total number of unexplained deaths, 27% were SGA. The most common specific finding in cases with known IUGR or SGA was an abnormality of the placenta, mainly changes suggestive of maternal vascular malperfusion. A significant association was found between increasing maceration and SGA and the longer the intrauterine interval, the greater the degree of fetal maceration and the greater the delta birthweight decrease. In addition, on average, fetuses lost 12% of their birthweight in the interval between delivery and autopsy. Without adjusting for such factors, using birthweight or bodyweight alone will erroneously significantly overestimate the role of SGA as an underlying factor in stillbirth causation.

Organ weights

Organ weights were significantly affected by maternal factors as well as the process of fetal maceration, the cause of death and the underlying mechanism for demise. The Brain:Liver weight ratio can be erroneously increased simply due to the effects of maceration rather than underlying pathology and hence such post death changes must be accounted for in clinical practise; on average the Brain:Liver weight ratio

should be reduced by 1 unit to account for the artefactual effects of maceration where present. The Brain:Liver weight ratio was significantly greater in cases of SGA with a placental cause of death compared to cases with birthweight < 10^{th} centile but with no pathological placental findings or with another cause of death. A Brain:Liver weight ratio of 5 can identify 'pathological' placental IUGR compared to other causes of death with a sensitivity of 73% and a specificity of 85%. A ratio of 6 improves specificity to 92% but reduces sensitivity to 55%. 'Pathological' IUGR can be distinguished from SGA cases with no placental abnormalities using a Brain:Liver weight ratio of 6 with a sensitivity of 53% and a specificity of 80%. Although of some use, the sensitivity and specificity of Body:Thymus weight ratios are not as effective as Brain:Liver weight ratios for the detection of 'pathological' IUGR.

Histology

The majority of fetuses had normal macroscopic and microscopic appearances of their internal organs after accounting for maceration and autolytic change. Cases of stillbirth showed a greater proportion of abnormal macroscopic or microscopic appearances but in the vast majority of cases, histological examination of internal organs did not provide the cause of death. The same was true for miscarriages. Less than 3% of deaths had abnormal microscopic findings that directly provided the cause of death and the majority of all histological abnormalities (68%) were seen in lung tissue. There were no cases in which histology of the spleen, adrenal glands, thyroid, thymus or pancreas provided the cause of death.

The Placenta

The placenta was submitted for examination in 946 (89%) of stillbirth and miscarriage autopsies. Nearly one third of all placentas had entirely normal

microscopic findings of the umbilical cord, membranes and placenta. In 208 stillbirths the placenta was abnormal (a significantly greater proportion than that seen in miscarriages) and could be directly linked to the cause of death (including those with diagnosis based mainly on clinical history, such as abruption). Nearly one third of all causes of death in the study were primarily placental; of these, 58% were ascending infection, almost always associated with midtrimester miscarriage and fresh intrapartum death, whilst 18% were related to specific histological abnormalities of the placental parenchyma; of which maternal vascular malperfusion accounted for the large majority (75%). Ascending infection was the major cause of late second trimester miscarriage with intrapartum or fresh stillbirth, specifically affecting women of black ethnic origin. Other placental histological abnormalities represented a significant cause of death for third trimester stillbirths, especially maternal vascular malperfusion and other specific lesions in the early third trimester. In an additional group of cases (78 of 1064, 7%) the placenta showed some histological findings that were abnormal but not considered severe enough to be attributed as the cause of death or were of uncertain significance (example patchy mild villitis). The majority of these otherwise unexplained deaths were macerated and had a similar gestational age, maternal age and ethnic distribution to the overall stillbirth population, including a predominant occurrence at or near term.

Thymus Histology and IUGR

This chapter assessed whether thymic involution could be objectively assessed and used to distinguish IUGR due to maternal vascular malperfusion form other causes. The Van Baarlen Grading system, when assessed blindly, was not associated with any particular cause of death group in stillbirth and was thus deemed an inaccurate tool in the assessment of IUGR. When assessed blind to all clinical history from

scanned images, there was no significant difference in the number of tingible body macrophages, Hassall's corpuscles or the average distance between lobules between groups. There was a significant statistical reduction in thymic weight and corticomedullary ratio, suggesting some evidence of accelerated thymic involution, in the IUGR case group, but with extensive overlap of all distributions.

Proteomics

In total, 305 proteins were successfully extracted from FFPE placental tissue and 250 proteins from liver tissue however; some of the proteins extracted could not be identified. Four placental proteins and two liver proteins were expressed in significantly different amounts between case groups. These protein yields are much smaller than those from fresh tissue and as such further research needs to continue to develop proteomic techniques in FFPE tissue. This Chapter does, however prove that proteins can be derived from fetal tissue in stillbirth and significant differences can be found between different case groups.

11.1 Limitations and Criticisms

One of the main limitations of this study was related to the retrospective nature of the dataset and hence intrinsic limitations regarding the extent of details of the antenatal history provided with autopsy reports. Some cases were submitted with no details of ultrasound scans or fetal birthweight and in others there was no maternal ethnicity or BMI provided. Such issues are common to all retrospective data series but this limited the number of cases that could be analysed within these categories and the effects of some risk factors may therefore have been over or underestimated. However, these issues were offset by the large size of the study; this study is the largest of its kind and therefore such effects on the study population are likely to be minimal. This study has highlighted that in 19% of cases, the cause of death was

clear on review of the clinical findings and external examination of the fetus. Within this study the aim was to use data and information that is currently available to assess the components of autopsy practise. However these findings suggest that if more information was provided it is possible that the proportion of unexplained deaths may be reduced. In view of this it is recommended that these study data be used as evidence to support possible changes in practise based on the findings of a future study to review the accuracy and completeness of clinical information provided within post-mortem packs compared to the original clinical notes. Furthermore, review of unexplained cases with full clinical information is required to determine whether this information affects the proportion of unexplained deaths or any other category of death.

In addition, a major strength is the use of predefined, objective definitions for all criteria used to ensure standardisation of interpretation. Where possible all data recorded was of findings present independent of the opinion of the reporting pathologist at the time. All classifications were performed identically for all cases. In addition, cases were included throughout the gestational age range from miscarriages through to post-term intrauterine deaths.

Cases were entered onto the database by one observer to further limit any effects of observer bias. All causes of death were reviewed by myself and my supervising Professor and allocated an objective cause of death thus limiting the subjective interpretation of the original autopsy findings by different pathologists and ensuring consistency.

Chapter 5 examined growth restriction and the effects of being small for gestational age in stillbirth. World Health Organisation (WHO) fetal growth charts were used as

a control measure of fetal birthweight. This provided some restrictions in the analysis of cases. Firstly, the WHO charts were not adjusted for the effects of maternal factors such as height or ethnicity, hence neither were the cases within this study. Secondly, the gestational age on the charts ranged from 23- 42 weeks and thus a percentage of cases within this study could not have a delta birthweight calculated as they were delivered prior to 23 weeks gestation. Growth charts are in existence that take into account maternal factors, (customised) however they are not freely available and detailed maternal demographic data were not available in all cases in this series.

In order to assess histology objectively, the images used to analyse thymic involution in Chapter 9, were scanned and hence of slightly poorer quality (compared to traditional microscopy slide assessment) for high power assessment of features such as tingible body macrophages, despite the use of a high quality digital scanner. In some high grade cases the distinction between the cortex and medulla was also impossible, making corticomedullay ratio calculation less reliable and when trying to assess the distance between lobules, the true angle would inevitably be different to that recorded due to the use of a 2D image of a 3D structure.

11.2 Evidence based guidance

Results from the present study can be incorporated into the six areas of current Royal College guidelines for autopsy investigation in fetal and perinatal death, providing the first evidence based guidance for stillbirth autopsy practise in the United Kingdom (159).

1. **External examination:** Current Royal College guidance recommended with the addition that the effects of maceration (its presence or absence), intrauterine interval and postmortem interval should be taken into

consideration when measuring body weight and assessing for SGA (Chapter 5).

2. **Internal examination:** Current Royal College guidance recommended with the understanding that the brain, liver, thymus, heart, lungs, adrenal glands and thyroid weights will be significantly affected by the process of maceration and intrauterine interval. The weights of the liver, spleen and thymus will be significantly affected in cases of SGA. Liver and thymus weights will also be significantly affected by placental causes of death. Brain:Liver weight ratio calculation is advised when assessing for the presence of true, pathological SGA, taking into consideration the effects of maceration, intrauterine interval and postmortem interval. In cases consented for limited autopsy only, Body:Thymus weight ratio can be used to assess for true pathological SGA with the knowledge that this, although useful, is less reliable than Brain:Liver weight ratio (Chapter 6).

3. **Placental Examination:** Placental examination remains a key investigation in stillbirth autopsy and current guidance should be followed. (NB: cord insertion site is of no particular significance and should not be over-interpreted by the reporting pathologist) (Chapter 7).

4. **Histology:** If consented for examination and a cause of death is not apparent from the clinical history or examination of the placenta, at most it may be suggested that one block of tissue should be taken of each lung and kidney, the heart, the liver and the brain. Histology of the spleen, adrenal glands,

pancreas and thyroid do not require routine histological examination as there is no evidence to support that such analysis will provide a cause of death (Chapter 8). There is little benefit in assessing for accelerated thymic involution (Chapter 9).

5. **Special procedures and investigations:** Current Royal College guidance recommended.

6. **Autopsy reports:** Current Royal College guidance recommended with consideration of the effects of maceration, intrauterine interval and postmortem interval on body and organ weights, particularly in the assessment of true, pathological SGA (Chapters 5-6). An objective cause of death should be sought to aid in the classification of deaths and comparison of deaths between centres (Chapters 4-9).

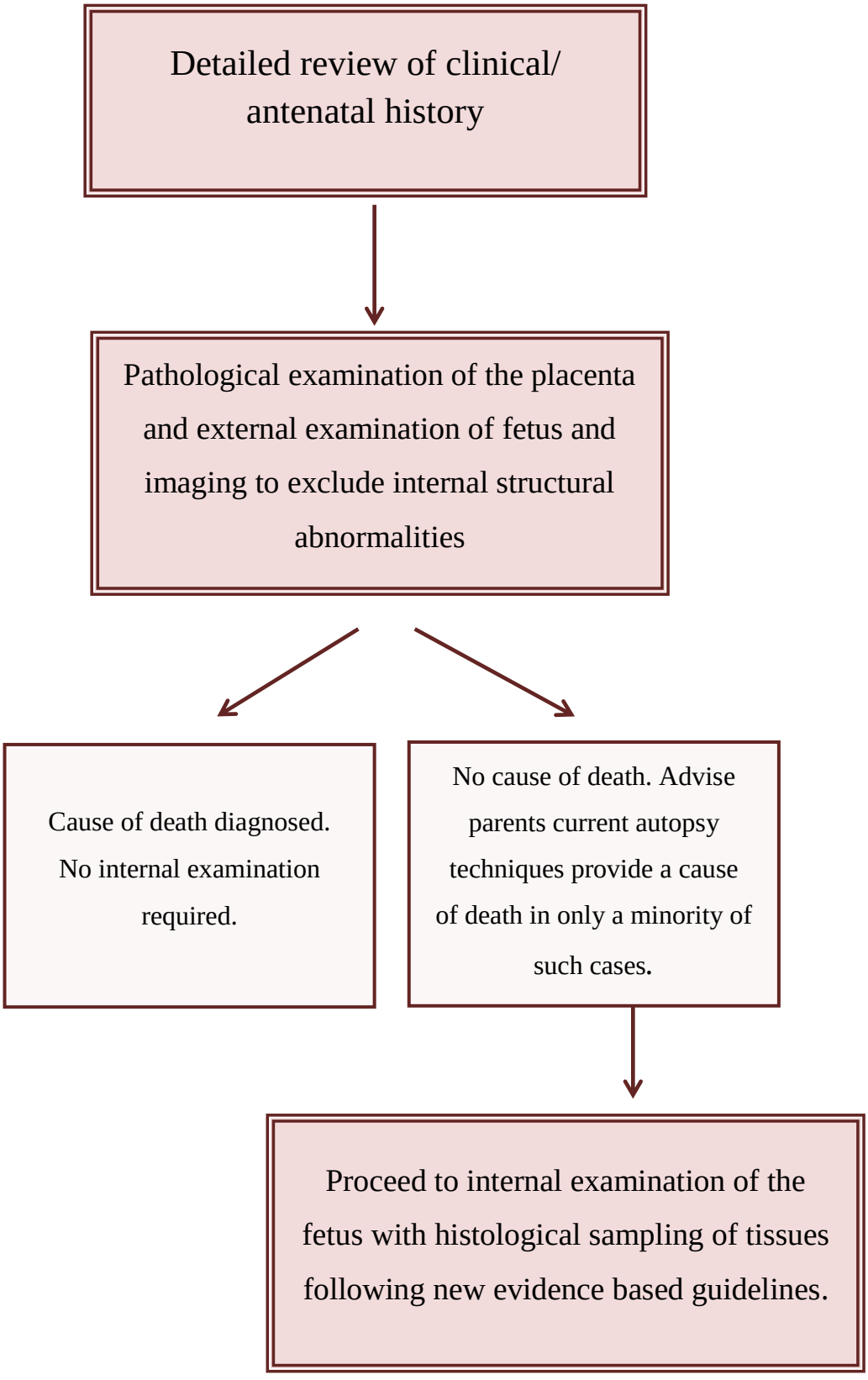


Figure 112 *Summary of evidence based approach to stillbirth autopsy practise*

11.3 Future studies

This study has utilised a unique and objective database to evaluate current stillbirth autopsy practise. Cases were predominantly third trimester stillbirths but also included a proportion of miscarriages to allow for the assessment of pathologies and associations over a range of gestational ages. It is the largest study of its kind and has provided statistical evidence for the improvement of clinical practise.

Nearly two thirds of all deaths were unexplained and nearly half of these deaths had no associated maternal, clinical or placental abnormalities. Of those deaths with associated abnormalities, little is known about the extent to which these abnormalities contribute to fetal demise and highlights the need for further research into the mechanisms of death in stillbirth. There is much debate about the relevance of IUGR and SGA in stillbirth and results from this study emphasise the importance of interpreting body and organ weights in light of fetal maceration, intrauterine interval and postmortem interval; findings that ideally need to be reproduced in other large scale studies.

The difficulty in classifying causes of death in stillbirth and the importance of remaining objective when diagnosing a cause of death was evident in this study. Further attempts must be made to unify classification systems in stillbirth in order to correlate findings not only between hospitals in this country but globally. The use of a united database for such deaths would prove beneficial for research, allowing for collaborations between centres of excellence as well as smaller centres where data may otherwise be poorly utilised.

Protein can now be successfully extracted from formalin fixed paraffin embedded (FFPE) tissue in stillbirths. Technical and genomic advances allow for the extraction of good quality proteins yields and for the analysis of protein function.

Histopathology departments hold huge collections of FFPE tissue, which if consented for research, could be a great resource of data collection both retrospectively and prospectively. Much research is needed on the usefulness and reproducibility of such investigations as well as interpretation of the findings and their relevance to mechanisms of death in stillbirth. The major area of future research in stillbirth investigation must focus on development of novel investigations to allow objective determination of cause or mechanism of death in the majority of cases which remain unexplained using current approaches. This is likely to require development of additional multiomic approaches.

The role of less invasive methods of investigation after death including post-mortem Magnetic Resonance Imaging (MRI) must also be highlighted. It has recently been reported that based on post-mortem MRI and non-invasive investigations; has a 95% accuracy compared to full standard autopsy; can reliably differentiate livebirths from stillbirths in relation to lung aeration; is able to provide accurate images of fetuses greater than 500g; is an accurate investigation for diagnosing significant neuropathology, even in cases in which tissue autolysis would usually prevent accurate neuropathological examination; and can identify clinically significant cardiac lesions and renal abnormalities (245-249). PMMRI is also favoured by parents as being more accepted than conventional autopsy (99% vs 60%) making it a useful investigation in stillbirth, particularly in parents who decline a traditional autopsy (250). Advances in PMMRI are likely to continue, highlighting the need for a continued multidisciplinary approach to stillbirth autopsy in which clinicians and researchers work together to strive for improved determination of cause of death in stillbirth.

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